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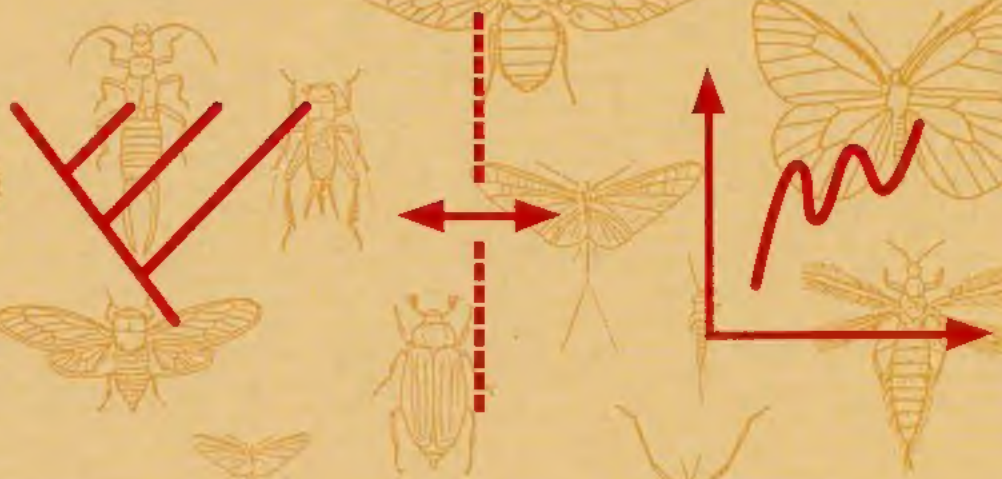
The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios



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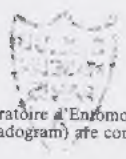

Cover: the biodiversity of Insects is suggested by the drawings of Gilbert HODEBERT (Laboratoire d'Entomologie, MNHN). On this background, the logical scheme of phylogenetic tests of evolutionary scenarios is presented: The phylogenetic patterns (the cladogram) are confronted to evolutionary models (the diagram) with respect of the principle of independence (the dashed line).

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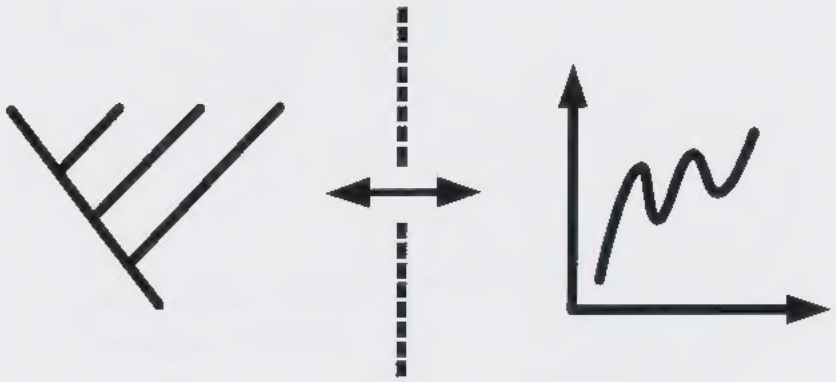
The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios



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MÉMOIRES DU MUSÉUM NATIONAL D'HISTOIRE NATURELLE

TOME 173
ZOOLOGIE

The Origin of Biodiversity in Insects: Phylogenetic tests of Evolutionary Scenarios

Philippe GRANDCOLAS

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Preface

"A major problem of contemporary evolutionary theory from an epistemological standpoint is not so much that its propositions are untestable, but that its main practitioners use theory to explain away pattern .. "

(ELREDGE & CRACRAFT, 1980 14)

For several decades and since the development and implementation of Hennigian methodology, phylogenetics has been revitalized and is now more and more incorporated into mainstream evolutionary biology Beyond the current use of buzzwords such as "biodiversity" or "tree-thinking", this shows that a new and promising research field in comparative biology has arrived Most comparative studies of these last years may be considered as either reconstructing parsimoniously past events or modeling population processes and extrapolating them to the past This volume deals mainly with the first kind of studies and emphasizes that both reconstruction and modeling are specific and complementary fields which should be kept independent and may thus test each other

Comparative biology searches for evolutionary patterns of descent with modification, which cannot be directly ascertained in populations but only reconstructed from the past Comparative biology has been recently rejuvenated by methodological advances in phylogenetic analysis These advances arose mainly by decreasing the use of *a priori* hypotheses to infer more reasonable and more refutable phylogenetic patterns Because phylogenetic patterns are constructed with minimization of *a priori* and *ad hoc* hypotheses, they can test the scenarios predicted by evolutionary models of processes which are extrapolated from population studies In this way, phylogenetic patterns do not provide direct estimates of the selective value of traits or direct explanations of processes but can possibly refute previous and incongruent hypotheses of selection or process

To carry out phylogenetic tests of evolutionary scenarios, it is thus necessary to understand clearly what are the respective aims and the pre-requisites of both phylogenetic analysis and process modeling Several contributions in this issue deal with contrasting the methodologies of these research fields PACKER stresses that using phylogeny is a highly heuristic task which may also help us to re-formulate evolutionary hypotheses WENZEL characterizes the extent to which phylogenetic patterns may be themselves evaluated and may permit the test of evolutionary models. MICHALAKIS, WAJNBERG & BERNSTEIN comment on principles of modeling and stress that the usefulness of models depends on their refutability GRANDCOLAS, DELEPORTE & DESUTTER-GRANDCOLAS contrast phylogenetic patterns and evolutionary models and emphasize that tests of congruence between them are highly heuristic MORAND challenges the view that modeling could not be used in phylogenetic analyses.

Phylogenetic patterns can be used to tell histories in selected groups of organisms or to test formerly hypothesized scenarios in these same groups They can be also used to test more general theories by repeatedly testing evolutionary scenarios predicted by these theories. This ultimate aim could be achieved using a large number of phylogenetic case studies to confer a statistical value to the addition of results of independent tests. This is why many studies of different groups with similar variation in the trait of interest should be carried out Most evolutionary models deal with hypotheses of stability and complexification in some particular traits These processes imply particular patterns of stasis and polarity which can be tested using phylogeny Also, many evolutionary models imply particular relations between traits These process-relations imply patterns of concentrated changes in traits which can also be tested using phylogeny Evolutionary models are tested here using phylogenies of water striders (N. M ANDERSEN), leafhoppers and planthoppers (T BOURGOIN), dung beetles (Y CAMBEFORT), vespid wasps (J M. CARPENTER), flies (C DAUGERON, L MATILE), crickets (L DESUTTER-GRANDCOLAS), scale insects (I FOLDI), cockroaches (P GRANDCOLAS), moths and caddisflies (N P. KRISTENSEN), sweat bees (L. PACKER) Evolutionary stability vs lability could also be tested with respect to the paradigm of actualism in paleontological studies As many past taxa remain forever unknown, probabilistic assumptions may be proposed to evaluate actualistic hypotheses (A NEL's contribution)

Unexpectedly, most studies do not corroborate former evolutionary hypotheses of stability and complexification Particular models of evolution were often shown to be flawed: wasp social parasites are not closely related to their hosts; the mutualism between ants and Homoptera is homoplastic, ground-dwelling Exoporia (Lepidoptera) are not primitive in this respect, predatory habits and mating swarms are not ancestral to empidoid flies; subsocial cockroaches are not forerunners of eusocial termites. As far as it can be reconstructed, evolution seems not to have proceeded straightforwardly, and shows many convergences and reversals This homoplasy was not predicted by models but is effectively detected using parsimony For example, social behavior in sweat bees, flightlessness in water striders, or loss of acoustic behavior in crickets all have reversed

Regarding the neodarwinian paradigm, this could either mean than genetic drift is more important than expected in some cases or that the hypotheses of generalized selective pressures are sometimes inaccurate Disruptive selection or genetic correlations could have influenced the evolution of traits which were hypothesized to be stable. This also stresses the importance of studies in developmental genetics (J DEUTSCH's contribution) some traits could be genetically determined in the way that they could have been lost and regained, producing similar phenotypes It should also be taken into account that these results can be biased regarding the whole history of life The ingroups selected for phylogenetic studies are necessarily relatively small and diverse and thus show probably more changes than expected in the whole (GRANDCOLAS *et al.*'s contribution) Nevertheless, one major conclusion of this volume is that homoplasy is detected even in supposedly stable and complex traits and should be now taken into account in the development of further evolutionary models

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Another important conclusion of this volume could be a methodological warning concerning phylogenetics and modeling in evolutionary biology. It should be emphasized that past reconstruction fundamentally remains conjectural. Conjectures may become either sound and refutable hypotheses, or gratuitous speculation. Only congruence among independent data sets gathered using minimal hypotheses permits us to propose sound evolutionary hypotheses. The use of *ad hoc* models (by analogy, by extrapolation,) must be definitely checked using appropriate "null" *and* minimal phylogenetic hypotheses. The need for minimal null hypotheses has already been emphasized in many research fields in evolutionary biology, with respect to hypotheses of adaptation (GOULD & LEWONTIN, 1979), biotic interaction (QUINN & DUNHAM, 1983), and biogeography (BALL, 1975). It should be stressed that this need also exists in comparative biology. Comparative studies must take this need into account, on pain to have their results flawed and refuted in the future.

This volume issued mainly from a Symposium (June 1996) organized by E.P. 90 CNRS (Laboratoire d'Entomologie, Muséum national d'Histoire naturelle) with the support of Réseau national de Biosystématique (financial support by Ministère de l'Enseignement Supérieur et de la Recherche, ACC-SV7). Many people have contributed to the realisation of this Symposium, thereby making possible the publication of this volume. I would like to thank the persons who have accepted to give talks and those who have provided moral and material help, J. NAJT, L. DESUTTER-GRANDCOLAS, S. TILLIER, C. CAUSSANEL, L. PACKER, J. BOUDINOT, E. GUILBERT, C. D'HAESE, A. SOULIER-PERKINS, O. MONTREUIL, A. BENDIB. Thanks also to Service des Publications of the Muséum national d'Histoire naturelle de Paris and to G. HODEBERT for his talented help concerning drawings. Referees have kindly accepted to read the manuscripts and certainly helped to improve them substantially: M. AKAM, N. M. ANDERSEN, A. BELLIDO, J. M. CARPENTER, J. CLOBERT, E. DANCHIN, P. DARLU, A. DEJEAN, P. DELEPORTE, C. DIETRICH, R. J. GAGNÉ, P. J. GULLAN, C. J. HODGSON, N. KRISTENSEN, D. MCLENNAN, D. R. MADDISON, S. MASAKI, J. MINET, C. M. NAUMANN, L. PACKER, C. PLATEAUX-QUENU, C. SCHOLTZ, P. TASSY, B. THORNE, J. VAN BAAREN, P. VERNON, J. WENZEL, B. WIEGMANN, S. WILSON, D. YEATES.

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Philippe GRANDCOLAS (Paris)

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The Relevance of Phylogenetic Systematics to Biology: Examples from Medicine and Behavioral Ecology

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ABSTRACT

Results of phylogenetic analysis are frequently used to investigate the pattern of evolution of characteristics of interest. In examples such as the evolution of spider webs, the number of horns on a rhinoceros or social behavior in halictine bees, the results of phylogenetic tests may lead to traditional views being overturned. However, conclusions based upon phylogenetic analyses of evolutionary pattern require careful consideration of character coding and taxonomic sampling as indicated by studies of rhinos and HIV respectively. Phylogenetic results are less often used to direct further research, an area of their application which remains underutilized. In this paper I concentrate on the application of phylogenetics to problems of social evolution in halictine bees. There are seven genera/subgenera that are known to contain both solitary and social species and at least 9 species which exhibit behavioral polymorphism with both solitary behavior and eusociality found within the same or different populations. *A priori*, these taxa would seem to be the best ones to use in tests of the selective advantages of eusociality. However, results of phylogenetic analysis indicate that in the majority of cases (*Halictus*, *Seladonia*, *Augochlorella* and *Augochlora*) it is solitary behavior that is the recent evolutionary innovation and eusociality is ancestral. Use of the non-phylogenetic approach to the comparative method in each of these instances would not provide information on origins of eusociality. In contrast, eusociality appears to be derived in both the subgenera *Lasioglossum* (in the species *L. aegyptiellum* for which the limited field-collected data are presented for the first time) and *Evylaeus*. Overall, of the nine species for which both eusociality and solitary behavior have been recorded, solitary behavior is the recent acquisition in at least 6 cases and the only probable case of recent origin of eusociality exhibited by behaviorally polymorphic species (*Lasioglossum (Evylaeus) comagenense* and *L. (E.) fratellum*) refers to origin of delayed eusociality. The application of phylogenetic methods to the study of evolutionary pattern suggests both which taxa are deserving of further field work and which require additional phylogenetic analysis.

RESUMÉ

L'intérêt de la systématique phylogénetique pour la biologie : quelques exemples issus de la biologie médicale et l'eco-ethologie

Les resultats des analyses phylogenetiques sont frequemment utilises pour inferer les sequences d'évolution de caracteres d'intérêt particulier. Dans des exemples tels que l'evolution des toiles d'araignées, du nombre des cornes de rhinoceros ou du comportement social des abeilles halictes, les resultats des tests phylogenetiques peuvent conduire à refuter des schemas traditionnels d'evolution. Cependant, les conclusions basees sur l'analyse phylogenetique des sequences evolutives sont dependantes du codage des caracteres et d'un echantillonnage taxonomique suffisant, comme cela est montre a propos des études concernant les rhinoceros et le virus HIV. Les resultats phylogenetiques peuvent être aussi utilises pour orienter de futures recherches, ce qui constitue un domaine de recherche encore trop peu explore. Cette article concerne principalement

PACKER, L., 1997 — The relevance of phylogenetic systematics to biology: examples from medicine and behavioral ecology. *In* GRANDCOLAS, P. (ed.), The origin of biodiversity in insects: phylogenetic tests of evolutionary scenarios. *Mem. Mus. natn. Hist. nat.*, **173**: 11-29. Paris. ISBN: 2-85653-508-9

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l'application de la systematique phylogenetique aux problemes d'evolution sociale chez les Abeilles Halictes Il y a sept genres/sous-genres qui sont connus pour regrouper non seulement des espèces solitaires et des espèces sociales mais aussi au moins neuf especes dont le polymorphisme comportemental englobe des comportements solitaires et eusociaux trouvés dans les mêmes populations ou dans des populations differentes *A priori*, ces taxa devraient donc parfaitement convenir au test des avantages que conférerait l'eusocialite en regard de la selection naturelle Cependant, les résultats de l'analyse phylogénétique indiquent que dans la majorite des cas (*Halictus*, *Seladonia*, *Augochlorella* et *Augochlora*), c'est le comportement solitaire qui est l'innovation évolutive et l'eusocialite qui est ancestrale Dans chacun de ces cas, une approche non-phylogénétique de biologie comparative n'aurait pas fourni d'information sur les origines de l'eusocialite L'approche phylogenetique indique que l'eusocialite est dérivee à la fois dans deux des sous genres de *Lasioglossum* (chez l'espece *L. aegyptiellum* pour qui des données de terrain sont presentees ici pour la première fois) et dans le genre *Evylaeus* Globalement, des neufs espèces chez qui l'eusocialite et le comportement solitaire ont été tous deux rapportés, le comportement solitaire est l'acquisition récente dans au moins six cas Le seul cas probable d'origine recente de l'eusocialite chez des especes au comportement polymorphique (*Lasioglossum (Evylaeus) comagenense* et *L. (E.) fratellum*) concerne une origine de l'eusocialite differee L'application de la methode phylogenetique à l'etude de l'evolution permet de déterminer à la fois quels taxa requièrent des études de terrain et quels taxa nécessitent des études phylogénétiques supplémentaires.

INTRODUCTION

In this paper I wish to explore the utility of phylogenetic systematics in providing answers to two questions i) what are we studying? and ii) what should we be studying? It may seem that answers to these questions are self evident – surely we always know what it is that we are investigating and are always confident that this is indeed what we should be studying However, recent reanalyses indicate that the confidence with which we approach our studies is often misplaced

What are we studying?

When biologists make comparisons of some feature of interest which varies between taxa we generally rely upon "common sense" arguments as to the adaptive value of the differences we observe Species A has some condition which is a result of adaptation to its environment whereas species B has some other state which is similarly adaptive We study how both characteristics function in their respective species/environments and understand both states to be adaptive However, the minimal requirement for a characteristic to be considered to be an adaptation is that it is a derived feature (CODDINGTON, 1988, 1995, GRANDCOLAS *et al.*, 1994, CARPENTER, 1997, this volume) In comparing states between the species one of them is likely ancestral to the other It is the evolutionary change between states that represents the results of selection and hence provides evidence for adaptation Consequently, we need to know the polarity of evolutionary change between character states, only then will we know what the direction of evolutionary change in the character of interest has been *i.e.*, only then will we really know what it is that we are studying

Results of phylogenetic analysis indicate that common sense approaches to polarity are often wrong For example, consider spider webs of the cob and orb varieties It is common sense to suggest that the rather untidy cobwebs made by some spiders served as an antecedent to orb webs, after all, they are apparently simpler in design and are constructed in places where it seems easier for spiders to negotiate web building However, phylogenetic analysis demonstrates the reverse to be true the orb web is ancestral to cobwebs with the latter arising several times independently in different spider lineages (CODDINGTON, 1988) Thus, in comparing cob and orb webs we would be answering questions about the selective advantage of cob versus orb webs

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whereas the common sense, non-phylogenetic, approach suggests we would be looking at the converse – the benefits of orb over cob webs

Another classic example, the evolution of horn number in rhinoceroses, has recently become more complex and illustrates the importance of careful character coding (DELEPORTE, 1993) A "common sense" argument is that as i) the protorhino condition would have zero as the number of horns along the midline of the head and ii) rhinos come with one or two horns, then the evolution of horn number must have followed the mathematically simple sequence of 0, to 1, to 2. However, a morphological phylogeny (GROVES, 1983) indicated unambiguously that one horned rhinos have evolved from 2 horned ancestors (CODDINGTON, 1988) Conversely, a recent molecular phylogeny (MORALES & MELNICK, 1994) is consistent with the 0 to 1 to 2 scenario in that one-horned rhinos form the first branch in the ingroup Combined data in a total evidence analysis (PACKER, unpublished) leads to various possible interpretations of evolutionary change in this character depending upon how horn number is coded If treated as an ordered character then there are two equally parsimonious resolutions of horn number evolution: i) from 0 to 2 (through 1) in the common ancestor, with a reduction to one horn in *Rhinoceros* (Fig 1A) or ii) from 0 to 1 in the ancestor followed by a change from 1 to 2 horns on two separate occasions (Fig 1B) As an unordered character, 0 to 2 to 1 is the single most parsimonious result (Fig 1A) This illustrates the problems that can arise as a result of alternative coding methods for the characteristics of interest, a point that will be returned to later However inclusion of fossil genera demonstrates clearly that among extant rhinoceros, loss of the frontal horn has occurred and so the polarity of change in horn number among extant rhinos is indeed from 2 to 1 (PACKER, unpublished)

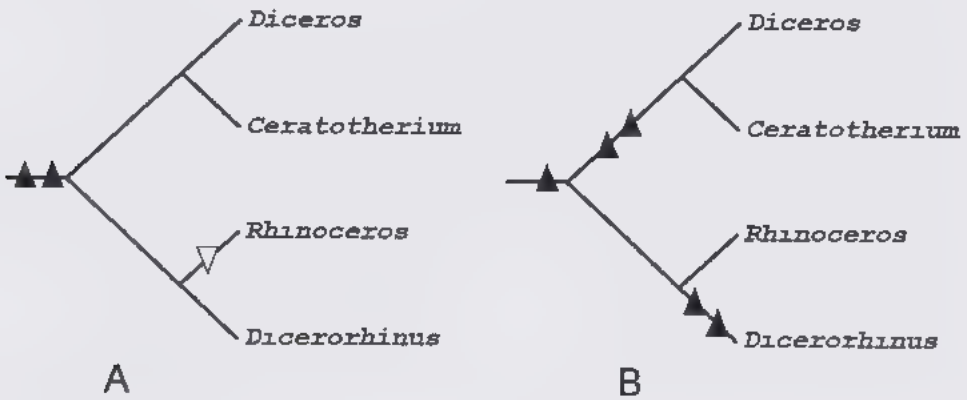

FIG 1 Evolution of horn number in extant rhinoceros. **A**: If treated as an unordered character then two evolutionary changes are required - from 0 to 2 in the ancestor and from 2 to 1 in *Rhinoceros* If treated as an ordered character, then three changes are required, from 0 to 2 (through 1) in the common ancestor and from 2 to 1 in *Rhinoceros* or **B**: from 0 to 1 in the ancestor with two independent derivations of the second horn

In much of modern evolutionary biology "common sense" is replaced by more complex models based upon population genetics or evolutionary ecology However, scenarios predicted

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by these sophisticated approaches can also be shown to be false using phylogenetic methods (ANDERSEN, 1997, this volume).

Although these examples stand out because their conclusions are counterintuitive, it is precisely this point that I wish to make - in the absence of a phylogenetic test of some *a priori* notion, one is likely to make mistakes by relying upon common sense biological "knowledge". These mistakes may lead to researchers spending a considerable amount of time (and money) asking the wrong question, a research program aimed at answering the question "what selection pressures caused the evolution of orb webs from cob webs?" would, at best, be doomed to inadequacy from the outset. For excellent accounts of the use of phylogenetic approaches and definitions and tests of adaptation see GRANDCOLAS *et al.* (1994) and CODDINGTON (1995).

What should we be studying?

Whereas mapping characters onto a phylogeny to verify or refute a scenario is becoming de rigeur in evolutionary biology (see most of the papers in this volume), the use of a cladogram to direct future research seems underutilized. Not only does phylogenetic analysis permit us to know the polarity of evolutionary change between character states, it also locates the position of the transition between states on the phylogeny. Clearly, comparisons of taxa on either side and in close proximity to this juncture are most likely to provide clues as to causation. These are the organisms that we should be studying, the comparisons that can most fruitfully be made.

The trouble is that our information is rarely complete. Phylogenetic studies of characteristics of interest usually cannot include all taxa because not all species are known for the traits of interest, phylogenetic information is incomplete, or both. There are several ways to overcome these limitations.

It may be possible to make an educated guess as to the state of some character of interest if a species is unknown behaviorally or ecologically but its position phylogenetically is known. For example, BROOKS *et al.* (1992) used phylogenetic analysis as a guide to field research and as a result discovered the breeding site of *Etheostoma wapiti*, an endangered fish species whose habitat requirements were not known. It is probable that discovery of the breeding site requirements of this species would have been delayed if it weren't for the application of phylogenetics to this problem. Thus, phylogenetic results can be used to guide field work.

Another approach is to produce a phylogeny for those species for which data of interest are available. With a phylogeny based upon a restricted sample of taxa it is still possible to trace the approximate position of a character state change of interest. Further systematic research can then add taxa to the phylogeny, concentrating upon those species thought to lie close to the transition point in the phylogeny. The results of this second phylogenetic iteration may then be used as a guide to which species should be the subjects of field research. This procedure greatly simplifies the problem of phylogenetic reconstruction, especially for speciose groups, although there is some potential for loss of accuracy when large proportions of a group are left out of a phylogenetic analysis. A more insidious cause of potential error with this approach is biased sampling of taxa. Consider some character state to be of great interest in comparison to the alternative condition: it is more likely that information on species possessing the interesting condition will be reported than data on the absence of the feature of interest. Species within higher level taxonomic groups will then have an unrealistic preponderance of the interesting condition and the results of mapping character traits onto the phylogeny will be biased in favor of

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optimizing the interesting character state as ancestral I will return later to an example of this in bees, but will first turn to a medical example

The Evolution of HIV

I will illustrate my point with reference to the evolution of HIV both because of the clear alternatives suggested by a phylogenetic approach and because it does serve as a great example for use in the classroom never will a class being taught the rudiments of phylogenetic methodology be so attentive as when the evolution of HIV is being considered

The classic story, that may be read in medical texts, is that HIV1 and HIV2 spread from non-human primates into the human population in Africa at some comparatively recent point in time and that they did this independently of each other yet more or less simultaneously (*e g* MYERS *et al.*, 1993), this pattern is referred to as the simian hypothesis Figure 2A shows a phylogeny consistent with dual transfer to humans The assumption that this disease is recent in humans is supported by the classic epidemiological dogma that diseases evolve from levels of high virulence to more benign relationships with their hosts The high virulence of HIV (HIV1 at least) is taken by many as being the result of relatively recent introduction of the virus into human populations by cross-species infection Combine several *a priori* notions into one logical argument and it is not likely that one will meet much opposition Fortunately, some researchers step aside from these assumptions and test the logical bases upon which they rest Here I will concentrate upon the potential role that different taxonomic sampling protocols may have played in this story For details concerning the history of other criticisms of the standard dogma over HIV see GRMEK (1990).

MINDELL *et al* (1995) have published a phylogenetic analysis of immunodeficiency viruses based upon sequence data (Fig 2B) The most parsimonious mapping of hosts onto the viral phylogeny suggests that the common ancestor of HIV1 and HIV2 was a virus that infected human beings and that, in addition to an initial colonization event into humans, there have been multiple independent interspecies transfers from human beings to other primates This suggests that HIV has had humans as hosts for at least several hundred years and that something other than a recent transfer into the human population is responsible for the extreme virulence of HIV1 (EWALD, 1994, MASSAD, 1996)

It is evident that analyses that leave out much of the diversity found within HIV1 and HIV2 are likely to bias the results in favor of the simian hypothesis MINDELL *et al.* (1995) took into consideration a great diversity of HIV and SIV lineages in comparison to previous analyses (*e.g* MYERS *et al.*, 1993) If one were to remove all but one of the HIV1 strains and all but two of the HIV2 strains from Figure 2B, using the same phylogenetic mapping logic, one would conclude that the common ancestor of HIV1 and HIV2 did indeed occur in a non-human primate and the simian hypothesis would thereby garner support simply as a result of biased sampling! This is precisely the taxon sampling protocol used by MYERS *et al.* (1993).

The currently available analyses do not suggest that a stable phylogenetic pattern for HIV SIV evolution and evolutionary changes in host use has not been attained by HIV researchers Nonetheless, it is clear that when a wide range of taxa are available, choice among them will influence the phylogenetic results obtained

Source MNHN, Paris

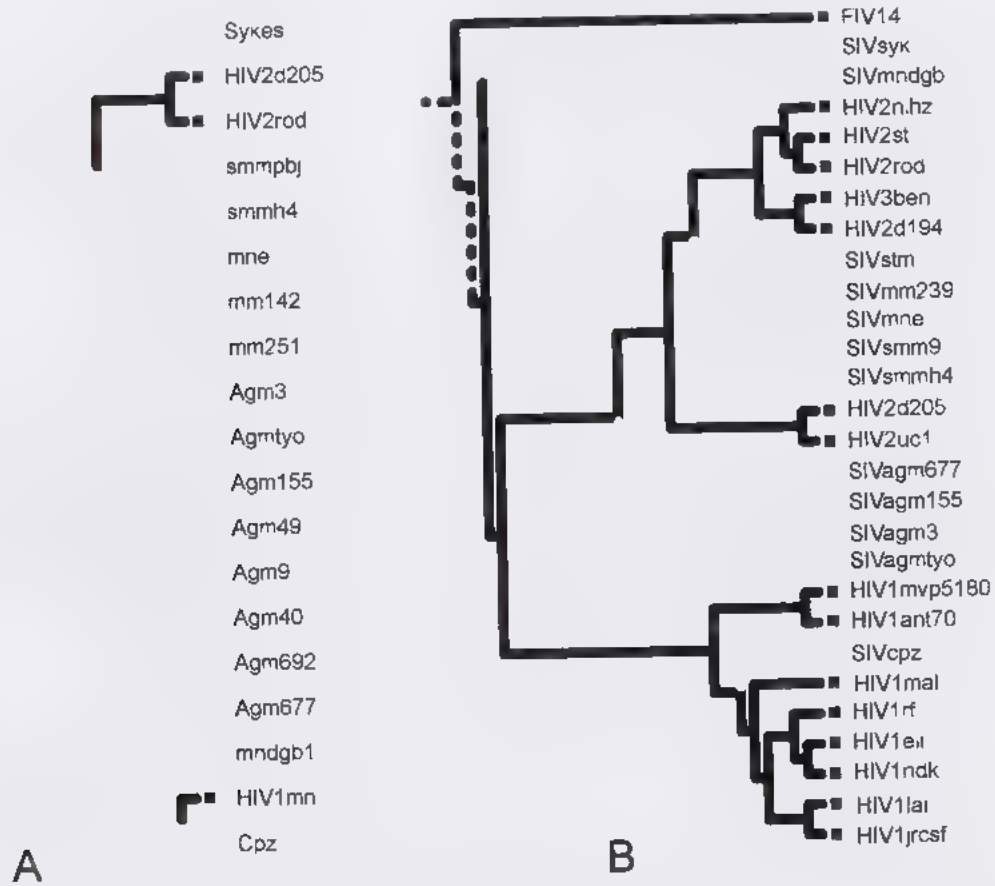

FIG 2 – Two scenarios for the evolution of host association for primate immunodeficiency viruses Pale grey patterns represent non-human primates as hosts, black ones refer to human hosts, dark grey ones represent feline immunodeficiency virus and dashed patterns represents ambiguous resolution **A**: the simian hypothesis is supported by MYERS *et al* (1993) who used only two strains of HIV2 and one of HIV1 in their analysis **B**: a human ancestral host, a more ancient ancestry and multiple infection into non-human primates are suggested by MINDELL *et al* (1995) Note that in both phylogenies different non-human primate hosts are not differentiated in cladogram shading Phylogenies redrawn from both sources

THE EVOLUTION OF SOCIAL BEHAVIOR IN HALICTINE BEES

Eusociality involves a reproductive division of labor between generations, archetypally between a mother queen and her worker daughters (WILSON, 1971, MICHENER, 1974) Among the Hymenoptera it is found in ants, vespid and sphecid wasps and various groups of bees

There have been many theoretical treatments aimed at explaining the origins of worker sterility (partial or complete) of which the kin selection (or haplodiploidy) hypothesis has received the most attention A fundamental prediction of this hypothesis is that female nestmate relatedness be high But, most analyses indicate this not to be the case (GADAGKAR, 1991) However, the vast majority of tests have concerned ants and vespine wasps, taxa which have been eusocial since the Cretaceous (BRANDAO *et al.*, 1989, WENZEL, 1990) Testing a hypothesis

Source MNHN, Paris

of origins of eusociality with these organisms is then somewhat too late, approximately 100 million generations too late Consequently, it has been stated that halictine bees are more suitable candidates for testing hypotheses of eusocial origins (PACKER, 1991, PACKER & OWEN, 1994) One reason for this is that all halictines exhibit primitive eusociality (*i e* they lack a marked morphological disparity between the castes) and with few exceptions (PLATEAUX-QUÉNU, 1959, SAKAGAMI & PACKER, 1994), their societies are annual Another reason for such optimism is the spotty taxonomic distribution of eusociality among the Halictinae Not only are there seven genera subgenera that contain both solitary and eusocial species (Table 1), there are at least 9 species which are known to have both solitary and social behavior as a behavioral polymorphism

TABLE 1 - Genera and subgenera of halictines which contain solitary and eusocial species Species which are both eusocial and solitary are listed under both categories Data from a variety of sources including YANEGA (1997) and MOURE & HURD (1987)

Genus / subgenus	Number of species		
	solitary	eusocial	unstudied
Halictus	4	11	80
Seladonia	3	8	>30
Lasioglossum	6	1	>60
Evylaeus	5	11	>60
Dialictus	many	many	100s
Augochlorella	1	3	13

TABLE 2 List of halictine species known to exhibit behavioral polymorphism either in the same, or different population * delayed eusociality and the normal annual eusocial colonies have both been reported ** eusociality known only as delayed eusociality

Halictus rubicundus	different	YANEGA (1997), EICKWORT *et al.* (in press)
Seladonia confusus	same	TUCKERMAN (pers. com.)
Seladonia tumulorum	same?	STOCKHERT (1933), SAKAGAMI & EBMER (1979)
*Evylaeus calceatum**	different	SAKAGAMI & MUNAKATA (1972)
*E. albipes**	different	PLATEAUX-QUÉNU (1993)
*E. fratellum***	same	VON DER HEIDE (1992), FIELD (1996)
*E. comagenense***	same	PACKER (unpubl. obs.)
*Dialictus problematicum***	same	SAKAGAMI & PACKER (1994)
Augochlorella striata	same	PACKER (1991)

(Table 2) By behavioral polymorphism I refer to a situation in which more than one type of behavior is routinely found within the species population under normal conditions, it does not refer to a situation in which accidents of mortality cause a change in social structure (for

Source: MNHN, Paris

example, if occasional early worker mortality leaves a foundress with no option but to act as a solitary female) If a population exhibits some eusocial colonies but there is a large proportion of foundresses that produce a brood with females none of which work then that would be considered an example of behavioral polymorphism Similarly if a species has populations that are social and others that are solitary it would also be considered to be socially polymorphic It should be noted that in no cases have these polymorphisms been unambiguously determined as having a genetic basis, although in one case bees from solitary and eusocial populations have been shown to retain their behavioral differences when reared under identical conditions (PLATEAUX-QUÉNU *et al.*, submitted)

Arguments for both the frequency and recency of eusocial origins in the Halictinae rest on the assumption that in each (or at least most) of the examples of intraspecific behavioral polymorphism or behavioral variation within a genus/subgenus, it is eusociality that is the derived condition But for each variable taxon this common sense approach to evolutionary polarity is a hypothesis that requires testing.

Below I will outline what we know of halictine phylogeny for each of the groups that are behaviorally variable In all cases our knowledge is doubly incomplete no single genus or subgenus has been subjected to a thorough phylogenetic analysis involving all species and for none of them is the behavior of all species known (Table 1) Given the large number of species in each genus/subgenus, it is doubtful that either of these areas of inquiry will be completed for any taxon in the near future Nonetheless, phylogenetic analysis can be useful in determining which taxa are likely to be most suitable for further study both in the field and phylogenetically

Methods

I have obtained phylogenies from the literature or from my own studies Wherever the example illustrates a point of general procedural interest, this is noted in the subheading

All phylogenies were either verified or obtained using Hennig86 and whenever multiple equally parsimonious trees resulted, successive approximations character weighting (CARPENTER, 1988) was invoked Inclusion of the social behavioral data in the matrix used to produce the phylogenies is not recommended in all but one case because of the difficulties associated with including polymorphic attributes (GRANDCOLAS *et al.*, 1994, DELEPORTE, 1993) in such analyses, especially when one might want to code behavioral polymorphism as an intermediate stage between solitary and social behavior (as would seem logical)

The examples

Total evidence and character coding in Halictus *and* Seladonia PESENKO (1985) provided a morphology-based phylogeny of species groups (as named subgenera) of *Halictus* and RICHARDS (1994) used allozyme electrophoresis to construct a phylogeny of many of the behaviorally known species in the subgenus and also some species of the subgenus *Seladonia* The former analysis is more complete in terms of the number of taxa included although it is now suspected that *Seladonia* (and *Vestitohalictus*) should be included within the ingroup (*Halictus*) rather than among the outgroups (PESENKO, personal communication) Clearly, behavioral data are not available for all of the included species and not even all of the species groups that PESENKO (1985) defines Nonetheless, mapping behavioral traits (Fig 3) onto the phylogeny

Source MNHN Paris

yields the conclusion that social behavior is ancestral and solitary behavior has arisen independently at least three times (PACKER, 1986)

The electrophoretic data of RICHARDS (1994) yielded two subsets of equally parsimonious trees and in both sets it is more parsimonious to treat eusociality as ancestral with solitary behavior and social behavioral polymorphism as being derived character states

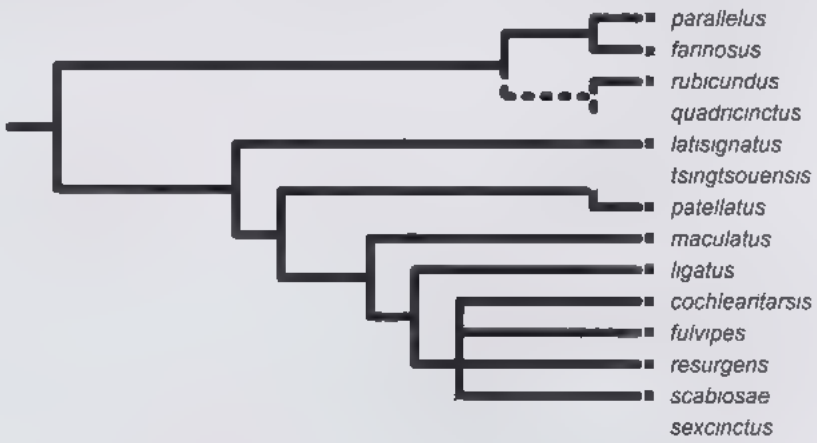

FIG 3 — Mapping behavioral characters onto a phylogeny for *Halictus* provided by PESENKO (1985) suggests eusociality to be ancestral with multiple independent origins of solitary behavior (from PACKER 1986) Pale grey patterns refer to solitary behavior, dark grey ones represent behavioral polymorphism and black bars indicate eusociality, dashed patterns represents ambiguity Polymorphism coded as a third state in an unordered transformation series

I have combined these data sets and added morphological data on *Seladonia* to produce a very preliminary "total evidence" phylogeny for the group In the analyses presented below, the electrophoretic variables were considered as unordered and the morphological ones were ordered where possible A smaller morphological data set than presented by PESENKO is used here as I have included only those characters whose polarity can be determined without reference to *Seladonia* as an outgroup and where character states for this subgenus could be homologized readily with those of *Halictus* I have taken advantage of some more recent behavioral research (EICKWORT *et al.*, 1996, TUCKERMAN, personal communication) in assigning behavioral character states to terminals

The results provided here are clearly far from complete even in terms of the representation of socially known species Nonetheless, they do indicate the importance that choice among alternative coding schemes may have upon deduced evolutionary scenarios Twelve equally parsimonious trees resulted from the analysis of the raw data matrix, each had a length of 159, a CI of 0 81 and RI of 0 77 One round of successive approximations character weighting led to one tree, the various statistics of which stabilized This tree retains both subgenera *Seladonia* and *Halictus* as monophyletic groups.

Three coding methods were employed for the social behavior data i) treating polymorphism as a third character state in an ordered transformation series ii) treating polymorphisms as a third character state in an unordered transformation series and iii) treating

Source MNHN, Paris

polymorphic species as having eusociality. These three coding protocols suggest different phylogenetic patterns for social evolution. When polymorphism is treated as an ordered intermediary character state social polymorphism is optimized as ancestral for *Halictus* + *Seladonia* and from this, solitary behavior is lost two or three times and eusociality is lost twice (Fig. 4A). When treated as an unordered three state character with polymorphism as the third state there is still substantial ambiguity (Fig. 4B). If the presence of eusociality is coded whether

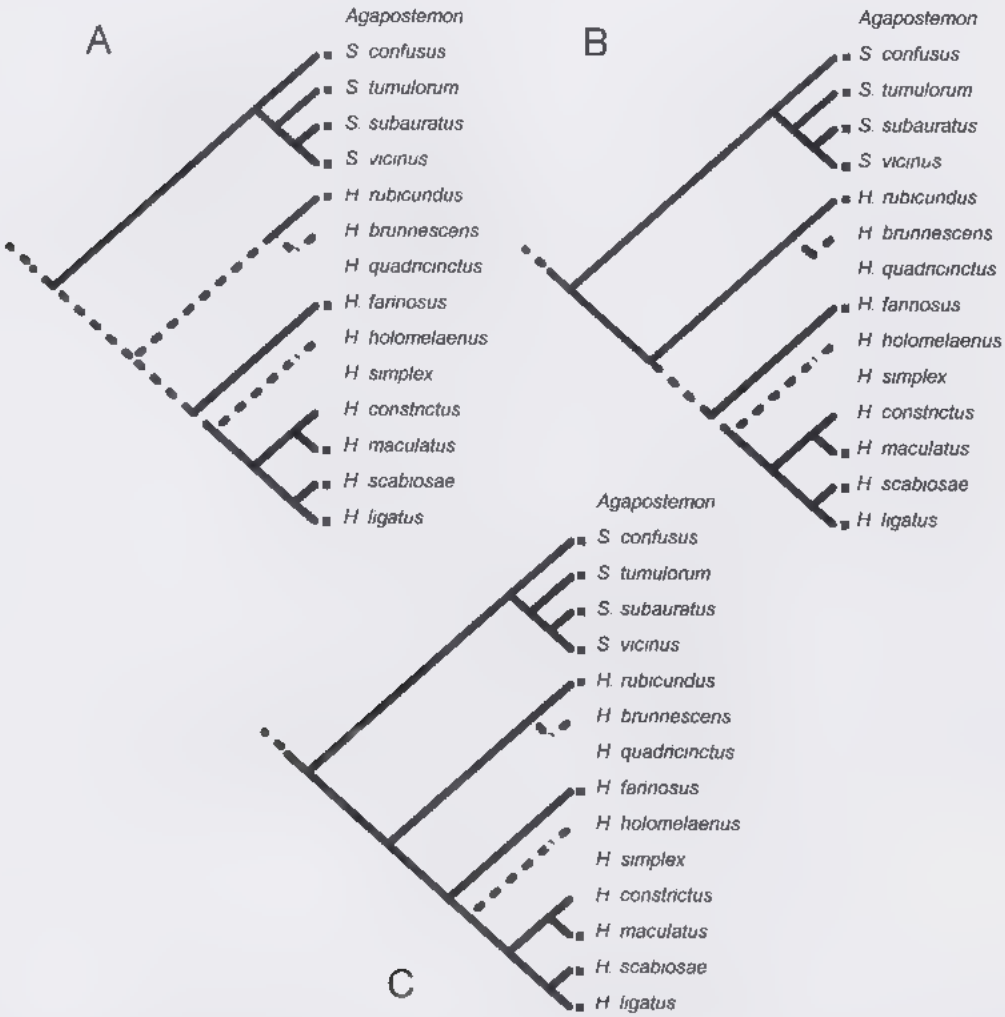

FIG. 4 — The scenarios for social evolution in *Halictus* and *Seladonia* combined with phylogenies based upon a combined data matrix from PESENKO (1985) and RICHARDS (1994). PESENKO's characters 1-6, 8-11, 17-23, 25-28, 35, 37 and 38 were included in the analysis with all multi-state characters coded as ordered except 1, 20 and 37. Note that *Agapostemon* is listed as the outgroup as this was the taxon used as such in the electrophoretic study, a much wider range of taxa were used to polarise the ingroup characters for the morphological analysis. **A:** Behavior optimized with polymorphism as a third intermediary state. **B:** polymorphism treated as a third state in an unordered character. **C** polymorphism treated as presence of eusociality. For explanation of cladogram shading see legend to figure 3.

Source: MNHN, Paris

polymorphic or not, then eusociality is ancestral with reversal to solitary behavior occurring at least twice (Fig. 4C)

The genus Lasioglossum *Lasioglossum aegyptiellum* (Fig 5) has been recorded as being eusocial based upon one nest excavated by KNERER I have had the opportunity to inspect the preserved contents of this nest, although KNERER's original field notes and dissection data have been lost The nest was excavated on August 1st, 1977 in the Camargue region of France Five adults were found within the nest (Table 3), all had well worn wings and mandibles although the largest individual had much the greatest amount of mandibular wear and, based upon the

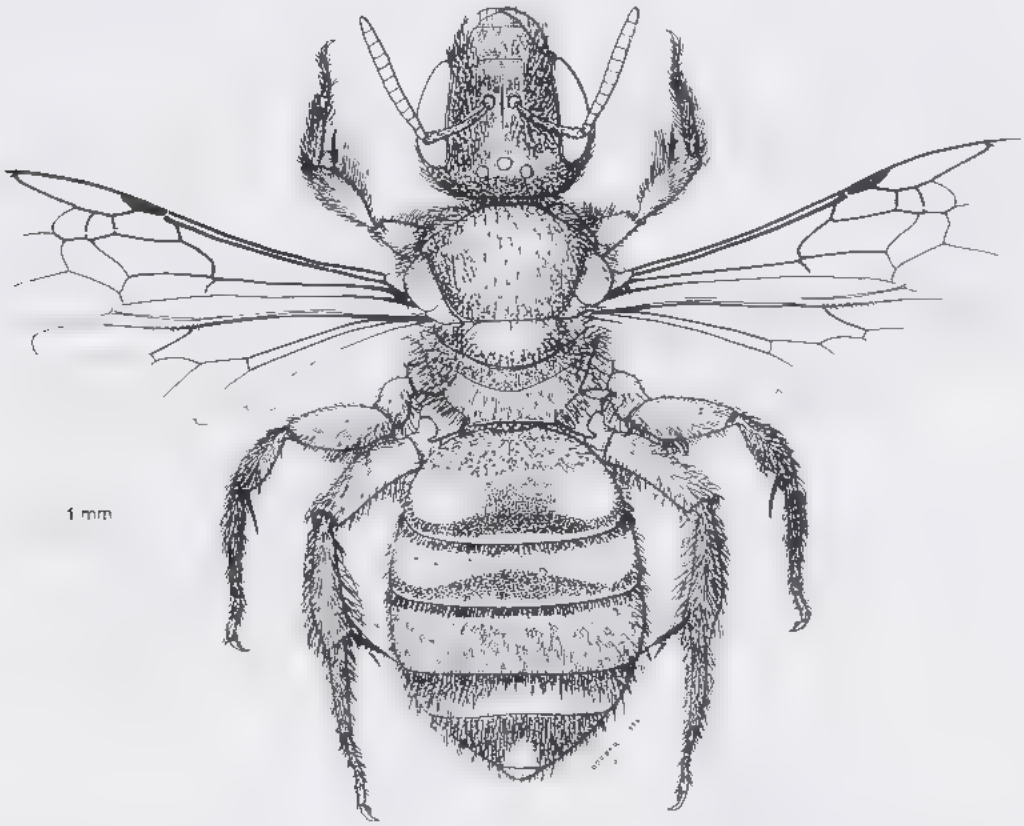

FIG 5 Habitus drawing of female *Lasioglossum aegyptiellum*, the species for which recent evolution of eusociality seems most readily documentable.

coloration of the wings, is the oldest individual among them, this individual is probably the queen All individuals had well developed ovaries, although deterioration and previous dissection of the specimens makes it impossible to provide accurate ovarian development indices or to establish which among them was mated Three male and one female pupae, five fully grown larvae and an unknown number of smaller larvae and pollen balls were also found The female

Source MNHN, Paris

pupa had the same head width as the largest adult female These meager data are consistent with eusociality with poor ovarian suppression in the workers

There is only this one species of *Lasioglossum s.str* which may considered to be eusocial, and at least 6 are known to be solitary (YANEGA, 1997) and so it is *a priori* probable that this represents a recent origin of eusociality (as also suggested by the high degree of ovarian development among the smaller adult females) Nonetheless, phylogenetic corroboration is needed There are currently no phylogenies for Old World *Lasioglossum* However, MCGINLEY (1986) has produced a phylogeny for the New World species, two of which (*leucozonium* and *zonulum*) are holarctic *Lasioglossum aegyptiellum* belongs to the *leucozonium* group (WARNCKE, 1975, PESENKO, 1986).

I have added *L. aegyptiellum* to the phylogeny by looking at its states for the 48 characters in MCGINLEY's (1986) data matrix As expected, *L. aegyptiellum* falls within the *leucozonium* group (Fig 6) based upon the synapomorphy of long male mandibles (a character which varies homoplastically elsewhere among New World species)

As both *L. leucozonium* (ATWOOD, 1933, STOCKHERT, 1933, PACKER, unpublished observations) and *L. zonulum* (STOCKHERT, 1933, KNERER & ATWOOD, 1962, PACKER,

TABLE 3 Data from a nest of *Lasioglossum aegyptiellum* * mandible wear is scored from 0 (unworn) to 6 (worn down to below the subapical tooth), wing wear is scored as the number of nicks in the wing margin, 'tattered' refers to the entire margin being abraded, 'very tattered' refers to the entire margin worn away such that wing length cannot be recorded

Female	Head width (mm)	Wing length (mm)	Degree of abrasion of* Mandible	Wing
1	2.8	6.9	5	tattered
2	2.7	6.5	3	10
3	2.7	6.6	3	tattered
4	2.7	?	2	very tattered
5	2.6	6.1	5	tattered

unpublished observations) are solitary, as are all other *Lasioglossum s.str* for which data are available (YANEGA, 1997), it is clear that eusociality in *L. aegyptiellum* is a derived condition (Fig 6)

Taxonomic sampling and Evylaeus PACKER (1991) provided a phylogeny of eight species of the subgenus *Evylaeus* based upon electrophoretic data Only one of the included species was known to be solitary and, at that time, another was known to exhibit behavioral polymorphism The results were unambiguous in demonstrating that the polymorphic species represented a recent reversal to solitary behavior in a montane population in Japan (SAKAGAMI & MUNAKATA, 1972) Whether solitary or eusocial behavior was ancestral to the subgenus as a whole remained

Source MNHN Paris

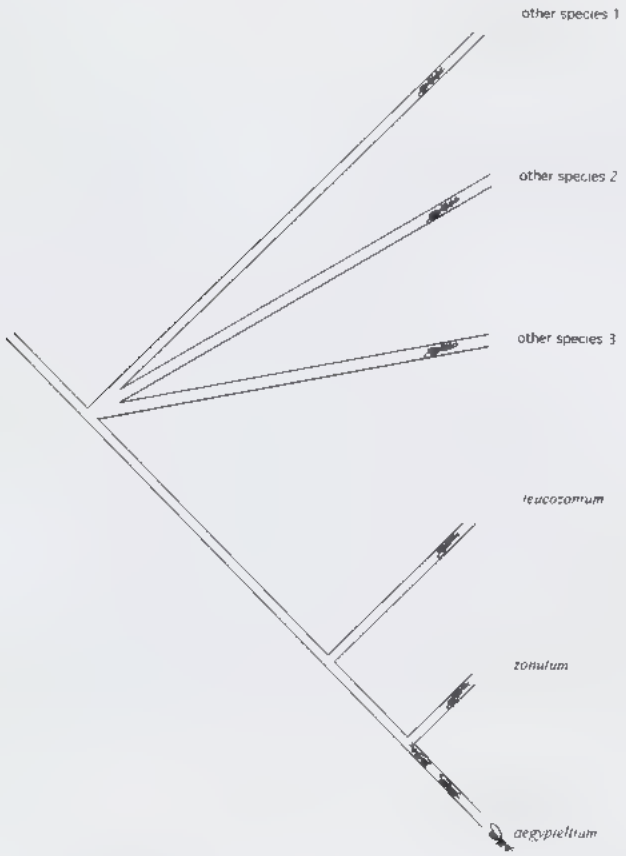

FIG 6 Phylogeny for three species in the *leucozonium* group of the genus *Lasioglossum* demonstrating that *L. aegyptiellum* has recently evolved sociality Data from MCGINLEY (1986) with additional information for *L. aegyptiellum* which has identical character states to those of *L. zonulum* for all characters used by MCGINLEY that could be assigned character states unambiguously

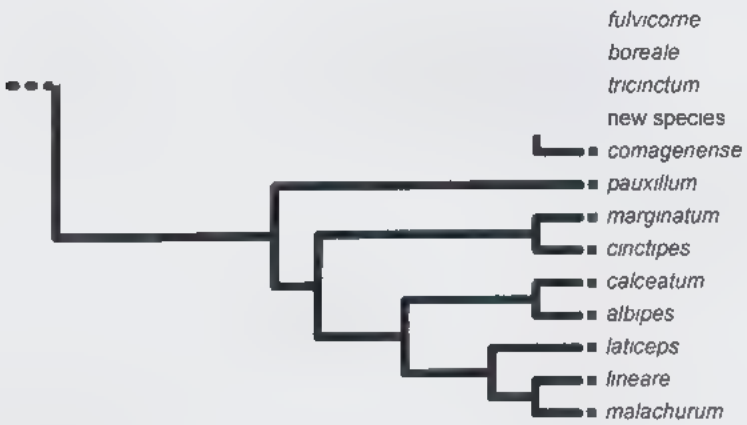

FIG 7 — Phylogeny for some species of the subgenus *Evylaeus* based upon a total evidence analysis (redrawn from TAYLOR, 1994). For cladogram shading refer to legend for figure 3

Source MNHN, Paris

undetermined, partly because the outgroup contained both solitary and social species Note that the fact that only one purely solitary species could be included in the analysis biased the result in favor of finding that eusociality is ancestral with only one solitary species in the ingroup and ambiguity as to the outgroup condition it is not possible to optimize solitary behavior as ancestral, this indicates the importance of taxon sampling when incomplete phylogenies and behavioral information are available Inclusion of the behavioral data into the data matrix used in phylogenetic analysis made no difference to the tree topology although CI and RI were reduced (PACKER, unpublished).

In order to resolve this situation additional information was required for taxa close to the root of the tree Several North American species are allied with *L (E.) fulvicorne* (SVENSSON *et al.*, 1977) and so some of these (and other species) were sampled and added to the phylogeny by TAYLOR (1994) In this case the results differ depending upon whether the behavioral data are used in tree construction or not The behavioral data considered appropriate to include in the phylogenetic data matrix are those that do not vary within species Thus, whether queen and worker size distributions overlap or not, whether fewer than one percent of the workers mate or not and three nest architectural variables were included whereas solitary versus eusocial behavior, multiple foundress associations and the number of worker broods were not included as these three features are attributes which vary within taxa One species (*L (E.) cinctipes*) changes position between electrophoretic and total evidence analyses The latter approach is preferred both philosophically (KLUGE & WOLF, 1993) and empirically total evidence places this taxon in a more reasonable position as judged by synapomorphies of the male genitalia (PACKER, unpublished data)

Four equally parsimonious trees were obtained with the total evidence data set with lengths of 125, CI of 0 84 and RI of 0 77 One round of successive approximations character weighting reduced the number of trees to three and the tree statistics stabilized at the second round to a length of 850, CI of 0 94 and RI of 0 92 The resulting Nelson consensus tree is shown in figure 7

Mapping behavior onto the total evidence phylogeny (Fig 7) leads to the following conclusions i) Solitary behavior is ancestral in *Evylaeus* with eusociality arising in the *pauxillum malachurum* clade and also, as delayed eusociality (in which founding females become queens in their second year of life after their first brood daughter(s) overwinter), in the lineage leading to *comagenense* ii) Social polymorphism is ancestral to the *albipes/calceatum* species pair (see PLATEAUX-QUÉNU, 1989, 1993 for data on the former species), (social polymorphism - with delayed eusociality and solitary behavior is also known from comagenense and it's sibling species *L. (E.) fratellum* (VON DER HEIDE, 1992, FIELD, 1996)) iii) It remains probable that the perennial societies of *L marginatum* are derived from an annually eusocial ancestral condition However, given the increasing documentation of delayed eusociality (a phenomenon which requires unusually detailed field work) This last conclusion may need to be revised This is because delayed eusociality is clearly a likely intermediate condition between annual and perennial colony cycles and it seems to occur, at least as a polymorphic attribute, fairly commonly.

As there are many more species in this subgenus that remain unknown behaviorally, a more broadly based phylogenetic study is required Once a more complete phylogeny including more of the taxonomic diversity in *Evylaeus* is available, it should be possible to predict which taxa

Source MNHN Paris

should be studied in the field in order to narrow down more closely the precise phylogenetic position of the origins of eusociality in these bees

The genus Dialictus Many species in this subgenus are known to be social and many solitary (PACKER, 1992, 1994, WCISLO *et al.*, 1993) These small bees are morphologically monotonous and very difficulty to identify, let alone analyze phylogenetically Nonetheless, molecular approaches to *Dialictus* phylogeny are underway (DANFORTH, personal communication) At present, it is not possible to say anything about the phylogenetic interrelationships among those *Dialictus* species whose behavior has been studied Nonetheless, it appears highly likely that recent transitions from solitary to eusocial behavior (as well as the reverse) can be documented within *Dialictus*

The genera Augochlorella *and* Augochlora A recent phylogeny of genera of the tribe Augochlorini demonstrates that the genera containing eusocial species form a monophyletic clade (Fig 8, DANFORTH & EICKWORT, 1997) *Augochlorella* is the sister group to the remaining genera and one of its species, *A. striata*, is behaviorally polymorphic at the northern edge of its range (PACKER *et al.*, 1989, PACKER, 1990) but only eusocial further south in New York (MUELLER, 1991) and Kansas (ORDWAY, 1966) As i) the Augochlorini are largely tropical American, ii) only 3 genera reach temperate North America, iii) *A striata* achieves a higher latitude than any other species and iv) other species of *Augochlorella* are eusocial (YANEGA,

FIG 8 Phylogeny of genera of augochlorine bees (redrawn from DANFORTH & EICKWORT, 1997) For cladogram shading refer to legend for figure 3 Note that for simplicity a clade of eight genera/subgenera the sister group to the lineage containing the eusocial taxa, is replaced here simply by the '*Megalopta* clade' Also there are an additional 13 genera subgenera more basal to the portion of the phylogeny shown here Redrawn from DANFORTH & EICKWORT (1997)

1997), it seems safe to assume that the solitary aspect of this locally polymorphic species is the derived condition and is presumably the result of the short summers experienced at the northern latitude on the edge of the species' range (PACKER, 1990)

The subgenus *Augochlora* includes the solitary wood-nesting *A. pura* (STOCKHAMMER, 1966) It is clearly nested well within the eusocial clade of augochlorines and thus is an example of the loss of eusociality

Source: MNHN, Paris

DISCUSSION

It is now no longer acceptable to make statements concerning the pattern of evolution in some characteristic of interest without reference to a phylogeny that corroborates that pattern (GRANDCOLAS *et al*., 1994). It is also clear that the incorporation of phylogenetic tests of the polarity of evolutionary change in specific characters often overturns dearly held convictions based upon *a priori* common sense biological "knowledge" This paradigm shift influences not only classifications (the original arena for the application of phylogenetic results) but also evolutionary scenarios

Cladistic methods provide a means of testing hypotheses of character evolution If the results indicate that the direction of evolutionary change in the character of interest differs from that traditionally held, then a lot of effort which would have been misdirected can be refocussed and events which actually happened can be investigated, rather than patterns which arose only as a result of the preconceived notions of biologists However, the utility of phylogenetics goes much further than this Particularly for the more speciose groups of organisms, basic biological data are generally not available for all species yet the sparse information that does exist can be placed within a phylogenetic framework If a phylogenetic analysis includes taxa for which information on the feature of interest is not available, the results may indicate clearly which taxa occupy crucial transitional positions in the phylogeny and are thereby those which should be studied in the field If both phylogenetic and behavioral/ecological information are fairly complete, then mapping the traits of interest onto the phylogeny will permit the pattern of evolutionary change(s) in the characteristic of interest to be discovered Thus, in addition to aiding in the interpretation of field research, through the establishment of polarity, phylogenetics can help us frame testable hypotheses as to how evolutionary changes took place and, through resolution of important areas of the cladogram, suggest to us which species are deserving of further study Thus, I would argue that phylogenetics is even more fundamental to many areas of biology than is statistics whereas statistics can tell us how to design experiments and analyze their results, phylogenetics can tell us precisely what changes have occurred in evolutionary history and which characteristics in which organisms are worthy of further study in the field or laboratory

In this paper I have plotted evolutionary changes between the character states "solitary" and "eusocial" for all groups of Halictine bees for which suitable information is available For the 6 genera/subgenera studied (no phylogeny is available for *Dialictus*), the solitary species are clearly derived from eusocial ancestors for four of them (*Halictus*, *Seladonia*, *Augochlorella*, *Augochlora*) There are four behaviorally polymorphic species known from among these taxa (Table 2) and for each of them, solitary behavior is likely the recent evolutionary innovation Thus, with respect to my first question "what are we studying?" any comparison of solitary and eusocial behavior within polymorphic species or between monomorphic species in these four genera would be analyzing those factors that promote solitary behavior

In *Evylaeus* all the primarily or purely eusocial species included here share eusociality from a common ancestor This represents a switch from solitary to eusocial behavior within *Evylaeus*, although the number of species derived from the social common ancestor is quite large

Source MNHN Paris

Phylogenetic study of this subgenus also indicates that two of the behaviorally polymorphic species have solitary behavior as the recent evolutionary innovation The remaining change to eusociality in *Evylaeus* occurs in the *comagenense/fratellum* species pair, both of which are behaviorally polymorphic within single populations which exhibit solitary, semisocial and delayed eusocial behaviors (VON DER HEIDE, 1992, FIELD, 1996) This is probably a comparatively recent evolutionary change Thus, comparisons among *comagenense*, *fratellum* and their solitary relatives could address the question of origins of delayed eusociality In the genus *Lasioglossum*, the only known eusocial species represents a recent evolutionary origin of eusociality

Thus, with respect to my second question "what should we be studying?" the following research directions are suggested First, phylogenetic and behavioral studies of additional species in the *L. leucozonium* group would be desirable Secondly, further phylogenetic and behavioral studies of relatively basal lineages within *Evylaeus* would be useful Thirdly, phylogenetic analysis of behaviorally known *Dialictus* species may be particularly important Furthermore, if research on the origins of eusociality is to be done, the genera *Halictus* and *Seladonia*, and the tribe Augochlorini (and vespine wasps, apine bees and ants) are best ignored

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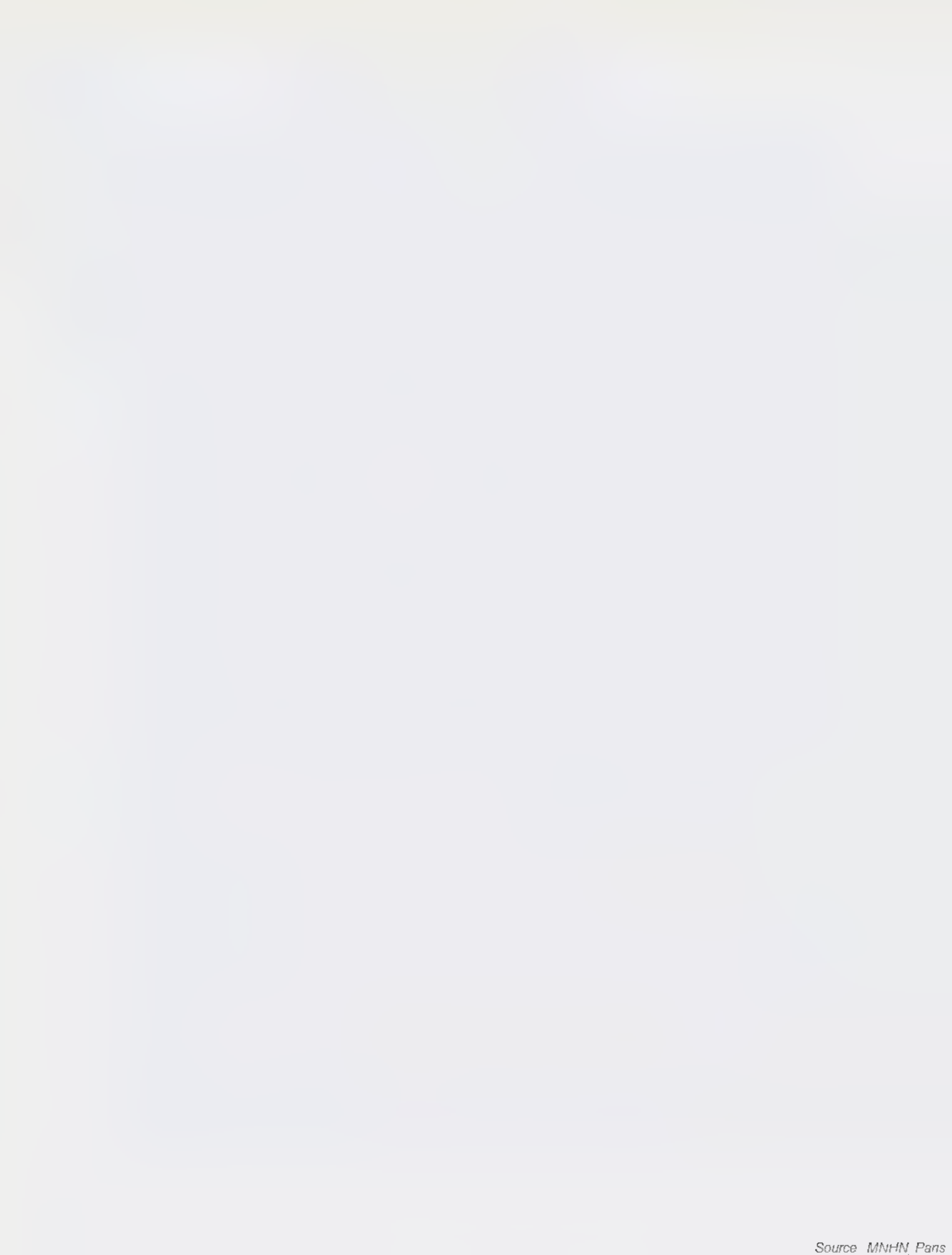
Source: MNHN, Paris



Source: MNHN, Paris

When Is a Phylogenetic Test Good Enough?

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ABSTRACT

Cladistic viewpoints have not been widely appreciated during the rapid growth of interest in phylogenetic tests of evolutionary scenarios. General opinion sometimes contrasts with cladistic perspectives with respect to the nature or severity of certain problems. Cladistic views are straightforward, if somewhat counter-intuitive in certain constituencies. The tree that best summarizes the data is always the most parsimonious tree (or consensus of most parsimonious trees) and nodes on this tree should not be dissolved based on low numerical support such as bootstrap values. Resolution of the consensus tree can be a problem when data are inadequate. Successive approximations weighting to derive better resolution is consistent with the cladistic paradigm in that congruence among characters determines the relative weights of the characters. This process is recursive, but not circular. In contrast, techniques such as maximum likelihood should not be used to derive a more resolved tree because that tree will not be based upon the data alone; results are biased according to (sometimes extensive) *a priori* dictations of the probable path of evolution and they will not reveal patterns incongruent with the initial assumptions. Construction of maximum likelihood trees relies on process theories unrelated to, and perhaps uninformative for, adaptive traits of interest. The characters of interest should be included in the final analysis because they are the data most relevant to the analysis. Claims that eliminating them improves independence or that including them leads to circular reasoning are incorrect both logically and empirically. Eliminating characters because they are expected to show high homoplasy is an unacceptable *ad hoc* protection of an hypothesis from a legitimate test. Most statistical treatments require unnecessary and often unsupportable assumptions regarding the process of evolution and expected distribution of traits. Character data and their appearance on the most parsimonious tree are preferred because they are the most free of assumptions and remain the most closely tied to the actual data.

RÉSUME

Qu'est ce qu'un bon test phylogenetique?

Le point de vue de la cladistique n'a pas été suffisamment pris en compte à l'occasion de l'interêt croissant manifeste pour les tests phylogenetiques de scenarios evolutifs. L'opinion generale est souvent en desaccord avec les perspectives cladistes au sujet de la nature ou de l'importance de certains problemes. Les points de vue cladistes sont clairs, même s'ils paraissent aller à l'encontre de l'intuition dans certains domaines. L'arbre qui rend compte le mieux des données est toujours l'arbre le plus parcimonieux (ou le consensus des arbres les plus parcimonieux) et des noeuds de cet arbre ne doivent pas être abandonnés parce qu'ils présentent de faibles valeurs de parametres numeriques, telles que des valeurs de bootstrap. La résolution d'un arbre consensus peut constituer un problème quand les données sont inadéquates. La ponderation successive appliquée à l'amelioration de la resolution est en accord avec le paradigme cladistique : c'est bien la congruence entre les caractères qui détermine les poids relatifs de ces caractères. La procedure est récursive et non pas circulaire. A l'oppose, les techniques telles que le maximum de vraisemblance ne devraient pas être utilisées pour obtenir un arbre mieux resolu, parce que cet arbre n'est plus basé sur les seules données : les résultats incorporent alors de manière parfois importante des opinions *a priori* sur le

WENZEL, J. W., 1997. — When is a phylogenetic test good enough? *In* GRANDCOLAS, P. (ed.) The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mem. Mus. nam. Hist. nat.* **173**: 31-45. Paris. ISBN: 2-85653-508-9.

Source: MNHN, Paris

voies evolutives les plus probables et ils ne reveleront plus des schemas incongruents avec les hypotheses de depart La construction darbres de maximum de vraisemblance repose sur des processus theoriques sans rapport, voire non informatifs, avec les traits adaptatifs a l'etude Les caracteres a l'etude doivent être inclus dans l'analyse finale parce que ce sont les donnees les plus pertinentes en regard de cette analyse Certains ont prétendu que leur elimination ameliorerait l'independance ou que leur inclusion conduirait au raisonnement circulaire ces assertions sont incorrectes à la fois logiquement et empiriquement Eliminer des caracteres parce qu'ils sont supposes être hautement homoplasiques constitue une entrave *ad hoc* inacceptable au test legitime d'une hypothese La plupart des traitements statistiques requièrent l'adoption inutile et souvent injustifiee d'hypothèses portant sur les processus evolutifs et la distribution attendue des traits L'utilisation des seuls caractères et leur apparition sur l'arbre le plus parcimonieux leur sont preferées parce que ce sont les procedures les plus independantes d'hypothèses *a priori* et qui demeurent au plus près des données réelles

INTRODUCTION

The modern systematist has a peculiar place in the natural sciences His discipline might be characterized as stuffing itself with data while trying to limit general theory The systematist collects information on morphology, development, genetics, phenology, behavior, ecological associations, biogeographic patterns, indeed, just about anything he can find is potentially useful for deducing hierarchical relationships of species and higher taxa But as for general theory, there is little more than the process of descent with modification, at least among Hennigian systematists (ELDREDGE & CRACRAFT, 1980 6) By contrast, nested or intertwined theories about the evolutionary process form the foundation of other fields, such as those concerned with competition or succession ecology, food web structure, sexual selection, or sociobiology In this light, it is interesting and ironic to see how recent enthusiasm for the primacy of phylogenetic perspectives has revitalized studies of theories about the process of evolution "Adaptation", one of the central features of Darwinian evolution, has earned the most attention The papers included in this volume are the result of a symposium that brought together a variety of systematists and others to discuss phylogenetic perspectives on certain evolutionary scenarios What distinguished this symposium from one that might have been on the systematics of many interesting organisms is that the speakers generally regarded the phylogenetic hypotheses as tools for understanding the process of adaptation (see also EGGLETON & VANE-WRIGHT, 1994, MARTINS, 1996) This method of examination leads to an improvement over the adaptive story-telling of old, but it also carries with it special hazards

The literature regarding phylogenetic tests of evolutionary scenarios is growing rapidly, but there seems to be a lack of proportional response from a Hennigian perspective on what are the procedures and problems in such an enterprise (but see CARPENTER, 1992b, CODDINGTON, 1994, WENZEL & CARPENTER, 1994) This paper may help to fill that void, or at least draw attention to certain issues that seem to deserve special consideration in this context One such issue concerns the intent of the study in the first place A systematist would generally make arguments about various character definitions, states, additivity, etc., and then derive a tree, but in the phylogenetic tests of adaptive hypotheses the process is reversed, producing a tree for the sake of deciding the details of transformations of chosen characters The phylogeny is only a tool, not a endpoint The relationships among taxa are the anvil upon which the traits of interest (and any conclusion regarding the process of evolution) are worked into shape This produces two problems which will be discussed in turn

Source MNHN, Paris

HOW GOOD IS THIS TREE?

The first and most obvious problem is that the best phylogenetic hypotheses are generally produced by those who see the phylogeny itself as the end product, but these are often not the people generating the hypotheses for the study in question. Taking care not to sound cynical, we should be cautious when someone who is not very interested in the phylogeny starts producing the hypotheses as a way to get on with his real work (hammering out those interesting traits). Of course, the hypothesis may be quite sound, but one cannot tell simply by reading that the relationships illustrated were derived by analysis with an approved computer program. Even if the primary data are beyond question, issues such as taxon sampling or character coding can have a great impact on tree topology, and even the best computer programs cannot overcome the errors introduced by a researcher's weak understanding of these issues. This problem is particularly important in these days when generating a few DNA sequences for a few taxa and making a tree from them is commonly done by people with no formal education in the sometimes deep problems of phylogenetic reconstruction. The solution, of course, is education.

Support

The simplest rule is to choose the optimal tree for the data in question. This approach has the advantage of logical consistency because all other trees are less well supported. This perspective leads us to the most parsimonious tree, or a consensus of equally most parsimonious trees (FARRIS, 1983, WILEY, 1981, BROOKS & MCLENNAN, 1991)(some non-parsimony based techniques will be discussed in *Resolution*, below). The strict consensus of several equally most parsimonious trees cannot be better resolved than any of the original trees, but the consensus should be considered highly supported anyway because *all* of the clades that appear are included in *all* of the multiple, competing trees. Whenever there is homoplasy, some parts of the tree may be more decisively reinforced by the data than others, but this is not a concern with respect to the optimality criterion of parsimony. By analogy, if we examined the relationship between body length and mass for humans, some points would be better predicted by a straight line than others, but we would nonetheless choose the least squares regression because it is optimal for the complete data set. The homoplasy in a most parsimonious tree can be compared to points that are displaced from a least squares regression line. Each represents an exception to the relationships we expect, but we use our optimum solution anyway.

Even when there is only one most parsimonious tree, measuring support is not straightforward. Ultimately, whether or not a clade is well supported depends on the characters that unite it. While experience and intuition are sometimes sufficient to make assumptions concerning the strength of morphological characters uniting a clade, DNA sequence data usually is not easily evaluated in a straight character by character comparison. What is the relative value of a change from "A" to "G" versus anything else? Also, the relative merits of alternative resolutions of homoplasy in DNA data are often not as logically interpretable as alternative resolutions of morphological homoplasy. The most widely used technique to measure support in this situation is the bootstrap (FELSENSTEIN, 1985), in which the original data are resampled randomly with replacement, new trees are generated from this new matrix, and the clades from the original tree are scored as present or absent on the new tree. This process is repeated and a score is produced for how often such original clades appear, with high scores (100% is perfect) suggesting that the clade is well supported, low scores suggesting it is not. Then clades with low

Source: MNHN, Paris

scores (90% might be a cut off point) are dissolved and only strong clades are retained on the "bootstrapped tree".

Objections to the bootstrap include that the statistics generated this way are not comparable to the normal probability estimates people want to use (SWOFFORD *et al.*, 1996 509), that competition for inclusion in the simulated matrices dictates that uninformative characters (such as autapomorphies) degrade clades when they ought to have no effect (CARPENTER, 1992a, KLUGE & WOLF, 1993), similarly, that strong support in one part of the tree is interpreted as weak support in another part, that asymmetrically branching clades derive higher bootstrap values than symmetrically branching clades even if the relative support for all clades is identical (M SIDDALL, pers com), and that clades with higher empirical content (more taxa) are penalized even when they have the same amount of support as a smaller clade (M SIDDALL, pers com) It is also important to note that the ordinary application of bootstrapping procedures is to derive a confidence interval for the estimate of a parameter of a distribution (MANLY, 1991 28), and that statistical mean and variance do not apply to unique historical identity of the phylogeny (WENZEL & CARPENTER, 1994 80 ff) So, although the bootstrap values mean something, it is not clear what they mean exactly, and it is clear that they do not mean the same thing when compared across different trees It would seem that these objections would suffice to exile the bootstrap from its current place of honor

An alternative to the bootstrap is BREMER support (BREMER, 1988), a technique that also gives values for each clade, but these are not intended to serve as statistical confidence tools Although the calculations are tedious, the principle is simple start with the most parsimonious tree and then find the shortest trees that do not contain each of the clades in the most parsimonious tree Because all other trees will be longer, steps will be added when a clade in the most parsimonious tree is broken up Each clade is then recorded as having support according to the minimum number of steps it costs to break it up A high value is better support, meaning many characters are *less* parsimonious on the shortest tree that does *not* contain the clade, a low value means few steps are added by interrupting the clade The problem with documenting BREMER support is that calculations can be prohibitive because of the necessity of calculating so many less-than-optimal trees The advantage of BREMER support is that values are more easily interpreted than the bootstrap values because they relate directly to how far away from optimal (most parsimonious) the tree would be if it did not contain the clade in question Interested readers will find a more thorough discussion of the relationship between these measures and short cuts to BREMER support in DAVIS (1995)

A common refrain in studies where trees are used to judge the validity of evolutionary scenarios is "maybe the tree is not good enough", or "maybe some of the branches are not well supported" From a Hennigian perspective, the most parsimonious tree (or consensus of parsimonious trees) is the best tree according to the data We may want to see values for bootstraps or BREMER support, but we should not change the tree based on these measures

IS THE TREE GOOD ENOUGH?

The second problem is related to the first one, but it is much older because it surrounds the merits of the phylogenetic hypothesis itself Phylogenies built for their own sake are usually compared to our previous understanding of the group with the intention of showing that we now know more than we did before Any increase in understanding is good However, if such

Source MNHN Paris

hypotheses are to serve as tools in another context, then a different kind of evaluation is necessary Gross polytomies may not matter depending on where they fall, so a phylogeny that says very little about relationships may still be "good enough" to answer some questions Figure 1A illustrates a case in which poor resolution of eight ingroup taxa has no effect on character argumentation In other cases, character distributions and exact topology can conspire to make even detailed understanding of relationships "not good enough" to answer the question Figure 1B shows a situation in which we cannot decide whether a character state was derived one, two, three, or four times among seven ingroup taxa, despite having a completely resolved tree

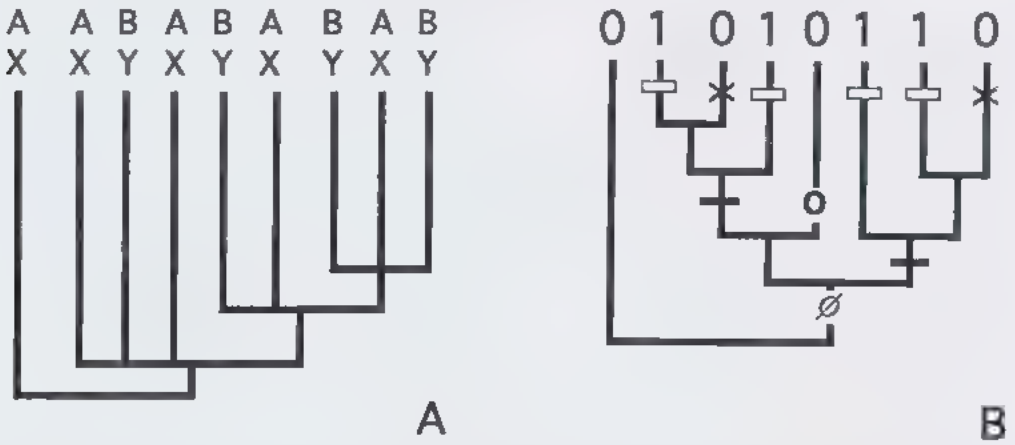

FIG 1 **A.** A poor tree that is good enough to answer the question Two characters exist in two states, (A or B) and (X or Y) There are many ways in which the three polytomies can be resolved and the two characters optimized but all require at least three separate derivations of the association of AX and BY This poor tree is good enough to answer the question of multiple origins and convergence **B.** A complete resolution of seven ingroup taxa that is inadequate to establish character argumentation The pattern of character states can be explained by four steps but it is ambiguous as to whether they represent one, two, three or four derivations of state "1" One scenario has all "1" states derived convergently (open boxes), another scenario has "1" states as two convergent synapomorphies for clades (solid tick marks) followed by two reversals (X) We can combine different halves of these scenarios to get two different ways of having three derivations and one loss (white boxes on one side combined with black ticks and X on the other) Another possibility has "1" as a unique synapomorphy for the ingroup taxa (o) with three subsequent reversals (X and ø) Our interpretation of the significance of character state "1" will change greatly depending on which optimization is chosen, but this excellent tree by itself is not good enough to resolve the problem.

Resolution

One reason strict cladistic techniques seem to be out of favor among many people is that a consensus of equally parsimonious trees often fails to resolve a polytomy Techniques can improve resolution, such as successive approximation weighting (FARRIS, 1969, CARPENTER, 1988), by which the characters acquire the weights suggested by their congruence with other characters (stability on the cladogram), a new tree is generated with the newly weighted characters, and the process is repeated until a stable solution is obtained This method provides a way to move from the initial set of trees to the local optimum (which may differ from the global optimum if the original search was too restricted) Critics have described these methods as

Source MNHN Paris

circular (SWOFFORD & OLSEN, 1990 499), but such an appraisal is clearly wrong because the weight of a character is determined by the degree to which it is coherent with *other* characters, and some analyses produce topologies that were not in the original set of trees (BROTHERS & CARPENTER, 1993), hence novel endpoints The process is recursive, but not circular

Other reconstruction techniques that do not rely on parsimony, such as neighbor joining or maximum likelihood, may produce one fully resolved tree when parsimony doesn't Unfortunately, the great shortcoming of these techniques is often the same as their strength They produce *only* one tree For example, when data support several alternative relationships equally, neighbor joining techniques (SAITOU & NEI, 1987) choose one arbitrarily based on the order in which taxa appear in the matrix (KLUGE & WOLF, 1993, FARRIS *et al*, 1996) This means the tree is not strictly determined by the data Neighbor joining is strictly an algorithm and has no optimality criteria at all, meaning there is no basis for a justification that the tree derived from a given study is somehow the best it can be In this case, basing additional work on one fully-resolved tree will be at least suspect and perhaps wrong In contrast, the multiple, equally parsimonious cladograms should not be seen as the failure of a divining rod in the search for water, but rather as the success of a child-proof top on a medicine bottle if you can't handle this, then you should go no further Cladistic analyses do not produce a definitive solution when data are ambiguous or lacking, and this result is most consistent with the general principles of the scientific method Unfortunately, the importance of multiple trees will not be widely appreciated as long as influential publications on phylogenetic methods simply ignore the issue entirely (*e.g* SWOFFORD & OLSEN, 1990; SWOFFORD *et al.*, 1996)

Judging from several conferences I attended recently, maximum likelihood is rapidly growing to be a popular method of tree-building, and it too can produce resolution or topology that is not present in the data matrix Many varieties of maximum likelihood estimates are used in modern statistical analyses, and more detailed discussions of their application to phylogenetic hypotheses are available elsewhere (SWOFFORD *et al.*, 1996), here I will approach only the issue of how the tree reflects the data used to produce it The basic operation, regardless of which maximum likelihood model is used, is that lessons about evolution learned in other studies are applied to the data in question to "improve" our understanding If other studies have shown that there is an evolutionary bias in the direction of mutation among nucleotide bases, then perhaps we should create a model of the process of evolution that will allow us to build a tree that accounts for this bias Supporters see this as a strength in that we are using our general knowledge to resolve some local problem, but cladists see this as a very serious flaw Maximum likelihood models favor some schemes over others *a priori*, and thereby dictate the path that evolution is *expected* to follow (hence, "likelihood"), and then evaluate the degree of likelihood *according to that path* Although most proponents of maximum likelihood readily acknowledge this, the severity of this shortcoming seems too easily overlooked Several critics have attacked maximum likelihood methods based on a number of weaknesses (FARRIS, 1986, WENZEL & CARPENTER, 1994), but here I will propose an additional flaw such methods are self-fulfilling and do not provide independent evidence of their legitimacy Trees built according to a given model cannot refute the model, and therefore it is clear that the model (which determines to some degree what we will find) is beyond testing If we decide in advance how evolution is likely to occur, we cannot later declare that we have discovered how evolution occurred Claims to special knowledge (say, that third positions evolve faster than the rest of the codon) become

Source MNHN Paris

dictations of pattern, the same pattern that is the point of the inquiry (WENZEL & CARPENTER, 1994). Indeed, arguing in favor of maximum likelihood, SWOFFORD *et al.* (1996: 428, 429) demonstrate that parsimony does not resolve placement of an ambiguous taxon, whereas maximum likelihood does based on the assumption that adding another step to a long branch costs less than adding a step to a short branch (that is, an expectation that more evolution probably occurs on a branch that is already "long" versus one that is already "short"). Thus, the fact that parsimony equivocates when data are equivocal is regarded as a flaw, but that branches that are already short (according to other data) are made to stay short (in the face of new data) is an advantage in maximum likelihood. Such logic would appear to condemn us to a science free from discovery. Some might say that weighting according to branch length is like successive approximations weighting according to homoplasy, but this is correct only superficially. Successive approximations takes over *after* all of the many parsimonious resolutions have been discovered, but maximum likelihood weighs things differently to get the first tree. Whereas parsimony allows all data to compete among themselves in whatever alliances form, maximum likelihood declares *a priori* that some data are favored and others not. Maximum likelihood can never contribute to the business of discovery as meaningfully as does parsimony.

Resisting attacks like that above, some argue that parsimony reconstruction contains its own assumptions. A recent incarnation of this view is that parsimony procedures assume at a minimum that the data are probably generated by a process that would allow parsimony to reconstruct the phylogeny correctly (SWOFFORD *et al.*, 1996: 426). Although this seems almost tautological, it is false nonetheless. Seeking the most parsimonious explanation for data at hand is a fundamental scientific principle and does not represent any statement or assumption about the process that produced the data. There is no assurance that the "truth" is obtained, only that there are infinity *less* parsimonious explanations, so we accept the optimal one and leave questions of "truth" out of it. If the data are misleading, parsimony will yield a misleading answer, and this is as it should be. Garbage in, garbage out. It begs at the margins of clairvoyance to claim that any method can be expected to give good answers from bad data. Strangely, SWOFFORD *et al.* (1996: 426) dismiss in a footnote the argument that parsimony is a fundamental aspect of scientific method, whereas they devote the next 50 pages to non-parsimony methods.

Despite how dismissive they can be about parsimony, proponents of maximum likelihood have been surprisingly forgiving of their method's grave flaws with regard to logical circularity, and quite generous in their acceptance of demonstrations purported to reflect problems in phylogenetic reconstruction. The current school arose, for example, from a model of highly variable rates of evolution with an absolute minimum number of taxa (FELSENSTEIN, 1978). Although some critics have argued that the variable rates of evolution are not an accurate reflection of what most data represent, it is even more obvious that the majority of reconstructions do not consider only four taxa, two of which are very different from the others, and therefore the exercise is highly contrived rather than representative. But, allowing themselves grace, the authors generate data from this model and then show that using this model to interpret the data gives the correct tree whereas parsimony does so less often. Thus, the demonstration is fundamentally circular in addition to being nonrepresentative of general problems in reconstruction. As an example, HULSENBECK (1995) generated artificial DNA sequence data, of lengths from 100 bases to infinity, for four (!) taxa to compare 26 reconstruction techniques. Data were generated from two models, JUKES-CANTOR (using equal base frequencies and one

Source: MNHN, Paris

substitution probability) and KIMURA Two Parameters, (using equal base frequencies, but two substitution rates as when transitions and transversion are unequal), and HULSENBECK measured the relative accuracy of different techniques for reconstructing the "true" tree As an example, when HULSENBECK used the KIMURA model to generate the data, KIMURA gave the best result, thus establishing the perfection of circularity The closely related JUKES-CANTOR model (see SWOFFORD *et al* , 1996, p 434 for relationship among these and six other models) was second best, demonstrating that logic that is nearly circular discovers itself almost as well (see summary in HILLIS *et al.*, 1996, fig 5) Despite demonstration that the parsimony methods performed well under all circumstances, and despite the fact that parsimony includes no elements of the model used to generate the data, and despite the fact that real evolutionary history is often not stochastic like his models, HULSENBECK concluded that maximum likelihood methods are preferred because they performed best Other than circular demonstrations of extraordinary problems, there is little to support maximum likelihood as the preferred alternative to parsimony

Eager to displace parsimony, proponents of maximum likelihood have been slow to produce the necessary studies of how their methods fare when data are *not* derived from the model used to reconstruct them (or more pointedly, not from any model at all), and comparing closely related models (as HULSENBECK did, above) hardly counts as a serious trial With respect to this charge, and much more relevant to the point of the current paper, maximum likelihood models are based on molecular evolutionary processes that have little relevance to the behavioral, ecological, or life history characters that people want to examine phylogenetically Ignoring the tautological (maximum likelihood) statement that more evolution is expected to occur on long branches, what assurance is there that the characters associated with the evolution of a new diet, or phenology, or habitat preference should obey the models of evolution based on, say, ribosomal DNA sequences? If defenders argue that maximum likelihood is better at finding the best tree, we should respond that the best tree will be found by including all the data, especially the characters of interest regarding diet, etc (see *Independence*, below), and then we will be including different kinds of data that can only be combined in a parsimony analysis Parsimony performs well at reconstructing histories in the absence of any knowledge of the evolutionary model, which is to say it will perform well even when we know no more about our characters than that they have evolved

Occasionally we will see a presentation in which the researcher has done both parsimony and maximum likelihood analyses to build trees to answer a particular question, perhaps "are polygamous males flamboyant" The two trees usually have similar branching patterns, which increases our feeling that they are valid The parsimony tree, typically less well resolved, may not be adequate to provide a definitive answer, and so a plea is made to consider the maximum likelihood tree Such a proposal, although born of necessity, is naive in the extreme If the parsimony tree shows that there are inadequate data to resolve the problem at this time, then that is the appropriate answer Defining a tree that is not specified by our data just to use it to answer the external question is irresponsible and dangerous Certainly, the researcher would not think of making up the other data (declaring, without any information, that a male was both polygamous and flamboyant), so why should he settle for a tree that is not determined by the data?

Source MNHN Paris

INDEPENDENCE

There will always be problems regarding the independence of data used in phylogenetic analyses The most obvious problem is that characters are used to make trees, trees are used to evaluate characters This problem reduces in part to questions of homology and character definition that are too large for this short paper Recent treatments from a cladistic perspective are available for the general issue (DE PINNA, 1991), and for morphological (PATTERSON, 1982), behavioral (WENZEL, 1992) or ecological (MILLER & WENZEL, 1995) data Here we will examine a subset of the general problem should we include the characters of interest in the phylogeny we will use to evaluate the evolution of those characters? I will argue that we should include the characters, and that intuitive fears of "circularity" are unfounded

The best test of an evolutionary scenario starts with having the best tree, a tree that relies on as much relevant data as possible What could be more relevant to the scenario than the characters in question? These characters would be considered useful data if the question was about *other* characters, so why should they be excluded now? This "total evidence" approach (also known as "combined" or "simultaneous" analysis), favored by many strict cladists, is rejected by some because of the idea that it is preferable to have an "independent" test, comparing the traits in question to a tree that was built without their contribution This widespread opinion has a strong pedigree (CODDINGTON, 1988, BAUM & LARSON, 1991, BROOKS & MCLENNAN, 1992, VANE-WRIGHT *et al.*, 1992) but it is wrong anyway As DELEPORTE (1993) states nicely, any problem of circularity is restricted to character coding (dictating transformations), not character choice, and therefore flaws are introduced prior to the analysis rather than through analysis itself This important point deserves more attention than it has received If our character coding is valid, then combined analysis will introduce no new error KLUGE & WOLF (1993) argued that the assumption of independence is the same whether comparing across data matrices or within a matrix No special independence is obtained by partitioning data into different sets, and so there is no circularity created by including all data in a single analysis Alternatively, if someone can partition any data set into two groups that disagree, are we then obliged to keep them separate forever? Clearly not Readers uninterested in epistemology might be convinced by reviews of the effects of combined analysis, often producing novel results not found in the partitioned data sets (BARRETT *et al.*, 1991, CHIPPINDALE & WIENS, 1994) Such novel results constitute empirical demonstrations that including all data does not result in circularity

Even in the spirit of independent sampling, there is no need to exclude the original observations that first suggested a relationship By analogy, when the first few male students enter a room and sit near the window, and the first few females sit near the door, it is not necessary to exclude these observations from the test to see if this is a general pattern The students who suggested the relationship are good data, as are the students who have not yet entered the room With this in mind, consider a more relevant problem several species of cave crickets are white, blind, and without circadian rhythm Perhaps they share these traits by descent (the hypothesis of homology), or perhaps these represent independent adaptation (rejection of homology) Combine these data with other characters and allow them to compete among themselves to build a tree If the cave species come out together, then this means that there is not enough information to reject the proposal that they are alike due to synapomorphy An obvious

Source MNHN Paris

question is "what if we get a different phylogeny when the characters of interest are left out?" Then we are still in the position of having an hypothesis of synapomorphy that is *not* rejected, because it still emerges in the combined analysis The hypothesis of synapomorphy survived the challenges of *all* our data, which is a strong test if we have a lot of other data In the absence of much other data, we may be subject to statistical Type II error (failure to disprove a false hypothesis), but that is different from circularity

Some readers may be critical of the cave cricket scenario presented above because synapomorphy is the null hypothesis, and failure to reject the null hypothesis is a weak statement Such critics may think that adaptation is likely to explain these traits, and that convergence is a more likely explanation than synapomorphy From this perspective, the null hypothesis would be that the common aspects of the cave crickets are independently derived Of course, the way to test this proposal is to return all data to the combined analysis and see if we find evidence for synapomorphy If the commonalties can be plotted as a unique synapomorphy, then we would have to reject the hypothesis of multiple convergent origins There is no logical way to avoid the combined analysis

Excluding the characters of interest produces the image of independence when we imagine that the other characters are a repetition of the phenomenon in question But they are not because they are *other* characters evolved from *other* pressures, and it is rare to see any two characters share precisely the same distribution among all taxa A tree built on other characters cannot be considered an independent "replicate" of the question we want to examine Statistically, eliminating the characters of interest is actually more like a jacknife procedure, in which we exclude some data to derive a pattern for the others Although jacknifing is useful and respected in general, it is not designed for the purpose of providing an estimate of the data that are *excluded*, and it is hard to imagine that anyone would recommend that the way to make sense of certain particular data is to exclude them from the analysis If the study centers on certain data, then they should be included in the analysis

Sometimes critics of the cladistic approach want to exclude the characters of interest because these characters are expected to be misinformation, as when independent origins are concealed by extensive convergence For example, WEST-EBERHARD (1996) endorsed a traditional hypothesis that social parasitism was derived multiply in the genus *Polistes*. Evidence in favor of the hypothesis of separate origins relies on observations of facultative parasitism (stealing nests) in ordinary species Such variation within original species could become extreme and permanent, eventually resulting in a new species, a social parasite To defend her position against a strong challenge (CHOUDHARY *et al.*, 1994, CARPENTER *et al.*, 1993), WEST-EBERHARD argued that covariation of morphological traits of interest, (heavy cuticle, square head, powerful mandibles) supply a false indication of synapomorphy (and a single derivation of parasitism) because these traits are expected be convergent adaptations and should have developed independently in each parasitic lineage Even if parsimonious reconstruction of characters indicates a single origin because the parasites are all closest relatives in a monophyletic clade, this interpretation is ruled out because it conflicts with the theory that parasites should evolve convergently (WEST-EBERHARD, 1996 315) In this case we must ask ourselves "What information would suffice to disprove the hypothesis of convergence?" If not the patterns among relevant data, then what? There is no good reason to ignore evidence offered by the characters of

Source MNHN, Paris

interest, and exclusion of them constitutes special protection of the hypothesis from a legitimate test Such a procedure is not within the scientific method.

The example above demonstrates a widespread view opposing combined analysis on the general grounds that "bad" data will lead us away from the better answer we could have had with the "good" data alone Most of these authors are proponents of maximum likelihood, which may explain why I do not agree with them For example, HULSENBECK *et al* (1996) offer a brief review and promote a method (HULSENBECK & BULL, 1996) to identify "pathological" data during reconstruction of the phylogeny for the four (!) taxa lizard, alligator, chicken, and mouse They argue that 18S rRNA sequence is more in conflict with other genes than is expected by pure sampling error, and that we should then keep the 18S data separate from the other data and come up with explanations about why the 18S data are different Aside from questions about how relevant such a study is to real phylogenetic reconstruction, one might well ask what we expect to achieve by combining (good) partitions that agree and separating (bad) partitions that don't (see also BROWER, 1996, for additional criticism of the method) The struggle between "good" and "bad" data is hard for me to understand because of the difficulty in knowing which data are "good" and which are "bad" As argued above, the business of discovery seems to be associated with patterns that were not expected (hence "discovery") which means they are "bad" data according to the maximum likelihood method More to the point, when carefully-examined "adaptive" traits are plotted on a phylogeny, it seems that they are relatively well behaved (see other papers in this volume), which demonstrates that adaptation does not confuse the larger pattern of evolution, and that cautious homology statements recognize independent origins of similar traits DELEPORTE (1993) is supported empirically if we are careful judging our characters, we do not create problems in reconstruction In situations where evidence indicates separate origins for a character of interest, it is appropriate to reexamine that character and see what differences might be found in the similar (but non-homologous) states Recursive examination is the best way to make use of information that was not available at the beginning of the study From the point of view of the phylogenetic analysis itself, such reappraisal commonly identifies homoplasy that is due to coding problems rather than real evolutionary problems, which is a necessary step to better understanding (MILLER & WENZEL, 1995) From the point of view of character of interest, reappraisal deepens our understanding of the nature and limits of similarity among separately derived states Character reexamination should be an integral part of all phylogenetic studies, whether focused on ecological transitions or not, or the process of discovery will be crippled pointlessly

STATISTICS

Many people become scientists because they like the idea of discovering things, knowing the answers to important questions, and deciding which of competing ideas is true Nature, however, does not always let us find a clear path to truth Some solutions to this problem were developed by early geneticists, who were forever presented with measurement errors of various sorts, and biology in general followed them to become more statistical Statistics is not only a way to summarize data, but a way to decide in favor of A or B when faced with some uncertainty It is only logical that the science of statistics has become associated with the uncertainty of phylogenetic reconstruction as it has with nearly all else in biology The problem with this new development is that a phylogeny is unique, it is an historical event that

Source MNHN Paris

happened once and cannot be resampled Whereas statistical analysis is good for predicting what sort of distribution or expectation we should get from tossing a coin (because we can sample and resample those events), it is poor for saying whether the fourth toss was heads or tails (because that happened only once) History is not a sampling problem, and efforts to make it become one are pseudoscientific at best In the context of phylogenetic tests of adaptation, WENZEL & CARPENTER (1994) and LEROI *et al.* (1994) discussed the inapplicability of certain procedures to phylogenetics, and readers would do well to review those arguments What is most relevant here is that a researcher can always make a phylogenetic test or reconstruction highly statistical, but would that actually make it better? The cladistic viewpoint is to rely upon the primary observations as much as possible

"Statistics As Truth" is a motto of many researchers who despise the idea of indecision There may be an overt advertisement for an artificial decision rather than an examination of natural data, as with this endorsement of arbitrary values substituted for real observations: " it does allow analysis of the data now, rather than waiting for actual phylogenetic information to become available" (GARLAND *et al.*, 1992 19, see WENZEL & CARPENTER, 1994, for treatment of other examples) Other researchers substitute statistics to produce an image of quantification when none is necessary LOSOS (1992) presented a statistical approach in which lizard ecomorphs from different island radiations were separated by principle component analysis (PCA) on morphometric values Then a phylogeny was used to reconstruct the PC scores for hypothetical ancestors and decide what ecological transitions occurred in the separate clades For this to be valid, the covariance matrix of all characters must remain the same through evolutionary time, which appears to me to rule out a lot of evolution, such as the evolution of species (whether ancestral or terminal) that are allometrically unique with respect to other species This assumption should be a source of concern, but for now let us overlook it In two radiations (Jamaica versus Puerto Rico) the transitions through PC space were from a twig-resting form to a tree-crown form to a trunk form, with grass and bush forms derived most apically, and it is reported that this is significant at $P<0.04$ (LOSOS, 1992 412) It is not discussed whether there would have been a different answer if we just reconstructed "twig", "crown", "trunk", and "grass", which seems to be the first thing to try Such a reconstruction would be based on the actual data of interest and would be free of assumptions about the process we are trying to discover If the patterns the author found are not supported by optimization of "twig", "crown", "trunk", and "grass", but rather due to the statistical reconstruction of hypothetical ancestors, then there is no evidence supporting his theory other than that it is consistent with the assumptions he made about the evolutionary process, that is to say it is not supported by the primary data If the patterns are supported by the primary data, then there is no point in all the statistical manipulation An example of completely needless statistics giving a clearly wrong answer can be found in COGNATO *et al* (1997) Examining the sex pheromone mixture of ten species of beetles, the authors asked if the pheromone variation is congruent with phylogenetic history or not The test consisted of a resampling of data from the original matrix (excluding the characters of interest) and evaluating the average homoplasy for these when plotted on a cladogram When the average homoplasy for the characters of interest proved to be higher than the average for the other characters, it was decided that they were *not* congruent with phylogenetic history Yet, four of nine pheromone components plot with no homoplasy, and three require only one additional step (COGNATO *et al.*, 1997 fig 1) Only two components seem

Source MNHN Paris

to be "poor", trans-verbenol and verbenone, both of which are suspected to be artifacts of unrelated chemical processes So, whereas the answer from plotting data on a tree is that at least four and perhaps seven components are congruent with phylogeny, the authors confused themselves into rejecting that proposal Fortunately, some researchers are content to plot data on a tree and let these patterns speak for themselves with no appeal to probability values (BASOLO, 1996, EMERSON, 1996, MCLENNAN, 1996)

EPILOGUE

It is inspiring to see that biologists of all sorts are interested in using phylogenetic perspectives to illuminate their evolutionary studies We must see that rigorous scientific methods are satisfied during the difficult task of discovering phylogenetic patterns Cladistic perspectives have been ignored sometimes because general eagerness to include phylogenies has produced a demand for single, well resolved trees, and unambiguous tests that are easily interpretable Unfortunately, these things cannot be delivered simply because we desire them Many methods or opinions that enjoy broad support among widespread researchers were not derived from careful consideration of their consequences or implications about how we do science Hennigian methods remain the most well-founded and will produce the results best suited to serve as the foundation of future research

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On the Utility of Mathematical Models and their Use in Evolutionary Biology

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ABSTRACT

Mathematical modeling is a powerful tool used in almost any kind of scientific endeavor. Mathematical models are as diverse as the problems we tackle with them because different problems call for different methods. The recent spectacular development of computing capacity has dramatically increased the number of potential applications of mathematical models since many more problems are now tractable numerically. Models can be characterized according to three essential properties: generality, realism and precision (LEVINS 1966). These properties often trade-off and only the questions to which the model is supposed to give an answer (*i.e.* evolutionary questions in the case of evolutionary biology) can guide the choice of the relevant degree of generality, precision or realism. Mathematical models can be a very useful tool in several respects. For instance, where intuition may be a poor guide, they can be used as guides to study qualitatively the behavior of very complex systems. They often help to clarify our ideas and their results can be used to build null hypotheses. Some caution is needed however in their use because it is often the case that several different models (or different combinations of parameter values of the same model) yield similar results compatible to biological observations. Considering alternative hypotheses is necessary and essential.

RESUME

A propos de l'utilite des modeles mathematiques et de leur utilisation en biologie evolutive

La modélisation mathématique est un outil puissant utilisé quasiment dans tout champ d'activité scientifique. Il existe une grande diversité de modèles mathématiques, des problèmes différents nécessitant l'utilisation de méthodes différentes. Le recent et spectaculaire développement des capacités de calcul en informatique a augmenté radicalement le nombre de problèmes auxquels on peut essayer de répondre. Les modèles mathématiques ont trois propriétés essentielles : généralité, réalisme et précision (LEVINS 1966). En général, il existe un antagonisme entre ces propriétés. Le degré de pertinence de chacune ne pourrait être guidé que par des considérations liées aux questions auxquelles le modèle est censé répondre. Les modèles mathématiques peuvent être utiles à plusieurs titres. Ils peuvent servir pour étudier le comportement qualitatif de systèmes complexes dans des cas où l'intuition est un guide peu fiable. Ils aident souvent à clarifier les idées et leurs résultats peuvent constituer des hypothèses nulles. Cependant, ils doivent être utilisés avec précaution. En effet, il arrive souvent que des modèles qualitativement différents, ou bien des combinaisons différentes des valeurs de paramètres du même modèle,

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MICHALAKIS, Y., WAJNBERG, E. & BERNSTEIN, C., 1997. On the utility of mathematical models and their use in evolutionary biology. *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mém. Mus. natn. Hist. nat.*, **173**: 47-52. Paris. ISBN 2-85653-508-9

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produisent des resultats similaires et compatibles avec les observations biologiques Ainsi, la prise en compte d'hypotheses alternatives est non seulement essentielle mais aussi necessaire

INTRODUCTION

Mathematical modeling is a very powerful tool used in almost any kind of human activity The nature of mathematical models is highly diverse because different problems require different methods The recent spectacular development of computing capacities and of software for mathematical computation has radically increased the number of potential applications of mathematical models, since many more problems are now tractable either by numerical calculations or by stochastic simulations

In this paper, we first give some reasons on why to build models, we then outline some characteristics of models and finally discuss some applications of mathematical models, especially in population and evolutionary biology Before proceeding any further we would like to warn the reader on several accounts First, none of us was trained neither as a philosopher nor as a mathematician, but rather as a population biologist This text should therefore be regarded only as a personnal account and opinion on what has become our main everyday activity We, by no means, intend to represent the orthodoxy or establish rules on how or why mathematical models should be built or applied, nor do we seek to be exhaustive Should the style of what follows seem dogmatic in some places, we would like to present our apologies to the reader in advance Such authoritative-like expressions just reflect our wish to avoid overloading the text with continuous repetitions of the fact that this text reflects only our opinion Second, we take it for granted that the reader is convinced of the utility of Theory in Science, actually of the impossibility to do Science without a theoretical framework Finally, citations to models or theories mentioned here should be solely regarded as illustrations of a particular point we want to make, and should by no means be viewed as a judgement, be it positive or negative, of their scientific merit

WHY BUILD/USE MODELS?

There are several good reasons to build mathematical models We will concentrate on the three that we think are most important in evolutionary biology Usually, models are built in order to test the effect of some changes in biological processes which can be evaluated this way In this respect, models often allow a conceptual synthesis A very good example is the development of theoretical population genetics in the first half of the 20th century by FISHER, HALDANE and WRIGHT (PROVINE, 1971) These three scientists, by developing mathematical models, managed to make the large majority of biologists accept DARWIN's theory of evolution, by showing that natural selection of random mutations was indeed able to account for observed patterns of adaptation What is really remarkable is that the development of this impressive body of theory took place while most of the underlying mechanisms concerning the way genes function or even what genes are, were largely unknown

While allowing a conceptual synthesis, mathematical models very often generate new hypotheses Such hypotheses can be subsequently theoretically or experimentally evaluated It is very often the case that the potential role of a particular mechanism has been ignored until

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revealed by a modeling exercise In other cases the relevance of a given mechanism as an explanation of a particular set of observations cannot be easily assessed experimentally. A model can suggest simpler ways to test this mechanism or at least help to explore its relevance

Finally mathematical models, in that they are often quantitatively predictive, can be very useful as aids to decision making and constitute null hypotheses against which predictions can be tested For instance, the development of the neutral theory of molecular evolution would have been impossible without the mathematical models developed by Motoo KIMURA and his colleagues (KIMURA, 1983) The mathematical models, in conjunction with statistical tests based on these models and developed by others, not only substantiated the plausibility of this theory, but provided a null hypothesis against which observations of molecular polymorphism are compared

PROPERTIES AND KINDS OF MODELS

It is possible to classify models with respect to three properties generality, realism and precision As LEVINS (1966) noted, there is usually a sort of trade-off between these properties For example, to be qualified as "precise" a model would have to take into account many detailed conditions and processes which apply to the situation modeled, most probably to the expense of other processes which do not apply to the modeled situation By doing so, the model would very likely be quite "realistic" as well, but it is highly unlikely that it would be "general" in the sense that it will probably not be applicable to a different situation without modification

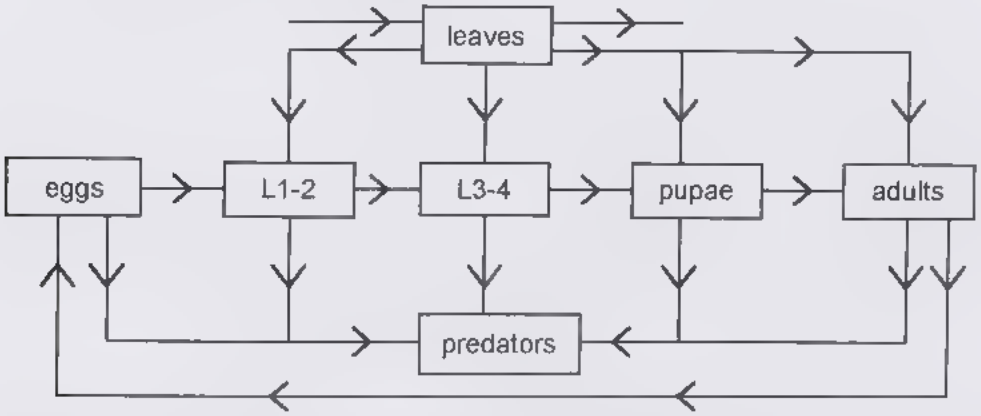

FIG 1 — Schematic representation of a tritrophic food chain involving a herbivorous insect (represented by its various developmental stages), its host plant (represented by its leaves) and its predators

The optimal mixture of these three properties can be defined only by the question addressed and the generality that one wishes the answer to have A given model will be very precise, perhaps too precise, to answer a given question and too general to answer another one To illustrate this point, consider the model depicted in figure 1 this model represents a tritrophic system consisting of host plants, herbivorous insects feeding on these plants, and predators feeding on the insects This model could be considered adequate to study the evolution of

Source MNHN, Paris

herbivorous insect populations It would be too detailed, however, if one wanted to address questions on tritrophic systems in general, since many tritrophic systems involve organisms which, for instance, do not have as many distinct developmental stages as insects On the other hand this model is probably too general for other questions one might want to address on herbivorous insect tritrophic systems For example, plants are only represented by the amount of leaves produced, assuming that all leaves are equivalent This is probably an oversimplification as quality of leaves of a given plant typically changes with leave age, while the quality of leaves of different plants can be very different Exactly the same arguments apply to the predators

STEPS IN MODEL BUILDING

The most important step in mathematical modeling in evolutionary biology is the very beginning identify an interesting biological question If the biological question is not relevant, then, obviously, anything that will follow will be equally irrelevant from a biological perspective (though may still be relevant from an applied mathematics perspective) Once the relevant biological question has been identified, one has to think of the different processes that might contribute to generate the observed patterns The next step would be the formalization in mathematical terms of the interactions of the various processes identified in the previous step This implies important decisions about which are the appropriate simplifications to be made Then, the mathematical model can be analyzed, analytically or by computer simulations, and the results interpreted and discussed Quite often the result of this analysis and discussion generates new hypotheses, most frequently due to the examination of further observations and the emergence of processes that might be involved and which had not be taken into account so far The activity of model building resembles to the construction of a spiral, where one starts with an initial idea which is reexamined in the light of the mathematical analysis and then expands to another idea by the incorporation of new elements

A step often discussed is the "validation" of a model. What is at stakes is not whether a mathematical model is mathematically correct (such cases are "relatively" easily resolved), but rather how does it apply to the biological question it is supposed to answer Such situations may arise for several reasons First, some relevant mechanisms may not be taken into account by the specific model In this case, the model as such cannot answer the question it was supposed to, but requires, at least, modifications We would like to express at this point the view that such "failures" are equally valuable for the process of scientific knowledge as "negative" experiments, *i.e.* experiments which fail to find statistically significant effects of the factors they have examined, and should therefore deserve equal attention A second reason for which a model may not answer a biological question is because some specific parameter values used to produce quantitative predictions with the model are wrong In this case, the problem is not actually due to the model but to its applications This kind of problem can be "easily", at least from the model's perspective, solved by implementing the model with new parameter values and does not require the modification of the model itself

In practice, of course, things are much more complicated than what would appear from the previous argumentation The first, and probably most important problem, is how one decides that a given model does not satisfactorily explain the observed patterns The answer to this problem is

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clearly question-specific If the question requires a quantitative answer one can conduct experiments and actually empirically measure the variables whose behavior the model is designed to predict Because quantitative variables can be easily accompanied by confidence intervals one may compare the values predicted by the model using the observed confidence intervals For questions requiring qualitative answers, however, it is much more difficult to decide whether a specific model provides a satisfying answer The main difficulty lies in the fact that the way the fit of the model is ascribed as "good" or "bad" remains largely subjective For instance, would a model designed to explain the evolution of dioecy in Angiosperms and which actually explains about 70% of the data be considered as validated or invalidated? Furthermore, the fact that models are not a single hypothesis but rather a full set of them makes it difficult to decide whether to just reject an unsatisfactory model or modify it

Another problem arises because many models may lead to the same patterns, at least for a particular variable Therefore the fact that a particular pattern can be satisfactorily explained by a given model should not be viewed as a confirmation of the model, but rather as the absence of an information The only way to avoid such caveats is to examine the alternative models and force them to produce contradicting predictions with respect to at least one experimentally measurable variable In saying this, we do not want to imply that scientists should not publish papers with mathematical models unless they have examined all alternative models We just suggest that until such an examination is done by somebody the question should still be considered open

CONCLUDING REMARKS

Mathematical modeling is a very powerful tool, which is necessary in almost any research program Models are necessary because they allow to build quantitative null hypotheses As evolutionary biology progresses towards the study of problems of increasing complexity the use of mathematical models becomes indispensable, simply because the increasing complexity of the questions addressed renders intuitive expectations untrustly and in some cases even impossible

Mathematical modeling offers a relatively inexpensive way to contrast alternative hypotheses and thus allow the evaluation of the relative importance of several mechanisms For such a parallel examination to be possible, alternative hypotheses should be forced to produce opposite predictions The interested reader can find more information on mathematical modeling applied in biology in BROWN & ROTHERY (1993) and in PROVINE (1971)

"Models" are used in many areas of human activities though they do not always bear the same meaning In the arts, a "model" is "what really exists", what the Artist wants to represent by her/his work In biology a "model" is meant to be a simplified representation of "what really exists" Such representations will only fulfill their role if they are guided by natural history, so that the final object of investigation remains clear in the mind of all actors

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Testing Evolutionary Processes with Phylogenetic Patterns: Test Power and Test Limitations

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ABSTRACT

Using parsimony, phylogenetic patterns may be inferred with cladistics, and may validate predictions issued from models of evolutionary processes. The use of parsimony is needed – whatever the evolutionary model implied – to minimize the number of unwarranted hypotheses, according to the elementary rules of comparative biology. Following this minimization, patterns are less hypothetical and more independent, and a higher number of evolutionary processes may be tested. One should be aware of possible biases in the comparison of the results provided by several tests in different clades, biases related to delineation of characters and ingroups.

RESUME

Le test des processus evolutifs par les sequences phylogenétiques : puissance et limitations du test

La phylogenie cladistique permet d'etablir par economie d'hypotheses des sequences d'evolution des caracteres. Ces séquences peuvent valider les predictions issues de modeles de processus evolutifs concernant ces mêmes caractères. L'usage de la parcimonie se justifie dans ce domaine, quelque soit le modele evolutif qui y corresponde, par la necessite de minimiser les hypotheses gratuites en biologie comparative. Il permet d'une part de ne pas rendre les résultats trop hypothetiques et d'autre part de ne pas oberer le test d'hypotheses supplementaires par manque d'independance. Il est recommande de prendre en compte les biais possibles dans la comparaison de resultats de plusieurs tests dans des clades differents, biais pouvant decouler de la définition des caractères et des groupes à l'étude.

INTRODUCTION

Phylogenetic tests of evolutionary scenarios formally existed since approximately twenty years (ANDERSEN, 1979). Following the development of cladistics, many people were interested in taking into account phylogenetic information for testing evolutionary hypotheses, as emphasized by several seminal papers (BROOKS, 1985, GREENE, 1986, CODDINGTON, 1988, 1990, CARPENTER, 1989). More recently, a large number of reviews dealt with this research field

GRANDCOLAS, P., DELEPORTE, P. & DESUTTER-GRANDCOLAS, L., 1997 — Testing evolutionary processes with phylogenetic patterns: test power and test limitations. *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mem. Mus. natn. Hist. nat.*, **173**: 53-71. Paris. ISBN 2-85653-508-9.

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(FUNK & BROOKS, 1990, WANNTORP *et al* , 1990, BROOKS & MCLENNAN, 1991, 1993, BAUM & LARSON, 1991, CODDINGTON, 1994, EGGLETON & VANE-WRIGHT, 1994a; MADDISON, 1994, SPENCE & ANDERSEN, 1994, MILLER & WENZEL, 1995, DESUTTER-GRANDCOLAS, 1996) The goal of these studies in comparative biology is to use phylogenetic patterns either to infer an evolutionary history *per se* or to test previous hypotheses of evolutionary processes (ELDREDGE & CRACRAFT, 1980; GRANDCOLAS *et al.*, 1994).

The number of available methods using phylogenetic information in the study of processes has also greatly increased (*e.g.* HARVEY & PAGEL, 1991, MILES & DUNHAM, 1993, HARVEY *et al.*, 1995, MARTINS, 1996) generally without clear distinction of their respective pre-requisites or uses (CARPENTER, 1992, GRANDCOLAS *et al.*, 1994) Only some empirical modeling studies have been carried out to evaluate and to compare these methods, and they did not settle general issues in this respect (*e.g.* GITTLEMAN & HANG-KWANG, 1994, WESTNEAT, 1995, BJÖRKLUND, 1995) Several works have also criticized the reliability of phylogenetic tests Regarding some specific evolutionary models, tests are supposed to be flawed either because parsimony is used or because adaptation is circumstantially detected (LEROI *et al* , 1994, FRUMHOFF & REEVE, 1994; GRETHER, 1995; SCHLUTER, 1995)

The phylogeny user who compares taxa and builds phylogenies for inferring or testing evolutionary histories could now wonder which method is the most powerful and relevant in his case study, the more likely to provide him with robust and reliable results He could also ask what are the limitations of these methods We try to answer these questions, focusing mainly on the phylogenetic tests of evolutionary scenarios which seem to us of prime importance regarding the aim of comparative biology.

TEST POWER

A test results from the contrast of two independent sets of data for instance, statistical tests compare an observed distribution and an expected distribution The phylogenetic tests of evolutionary scenarios compare phylogenetic patterns and patterns implied by evolutionary processes (*i.e.* evolutionary scenarios), to infer sound hypotheses of evolution (ELDREDGE & CRACRAFT, 1980, CARPENTER, 1989, GRANDCOLAS *et al.*, 1994) As in any test, if expected and observed data sets are incongruent, the hypothesis under test (which has been obtained using unwarranted hypotheses) is rejected as unsatisfactory Conversely, the congruence of the two data sets provides independent support (*i.e.* corroboration) for the unwarranted hypotheses used for obtaining one of the data sets By unwarranted, we mean hypotheses which are not substantiated directly but made by extrapolation or by logical reasoning

Phylogenetic tests may be ranked relative to other methods of extracting historical information, according to their respective testing power This testing power may be estimated with respect to the range of different situations in which the tests can be performed, and with respect to the ratio and the reliability of refutations which they can produce Estimating the testing power makes necessary to assess critically the kind of items to be compared in the test, the intrinsic properties of these items and thus the way to contrast them maximally Both the phylogenetic patterns and the evolutionary scenarios should be examined in this perspective, in order to draw the guidelines for carrying out the tests

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Lessons from the phylogenetic patterns

Minimizing the burden of hypotheses Evolution is a historical and unique phenomenon which occurred in the past and produced similarities and differences between taxa The aim of comparative biology is to fill the gaps existing between the taxa to understand their differences, using the principle of descent with modification (Fig 1) Consequently, comparative biology deals mainly with hypotheses, *i.e.* the basic hypotheses of descent patterns which link the respective characters' states in the different taxa (NELSON, 1970, FARRIS, 1983) These hypotheses will never be ascertained totally, because gaps in knowledge still remain (PATTERSON, 1994) Neither fossils nor additional taxa could provide anything other than hypotheses because these additional taxa could only insert themselves between other taxa without totally filling the gaps Consequently, any methodological advance in comparative biology should consist in decreasing as much as possible the number of hypotheses For reconstructing the past, one should not add any extra-hypothese (*e.g. ad hoc* hypotheses *sensu* FARRIS, 1983) to the basic and necessary descent hypotheses linking character states in taxa Any additional *ad hoc* hypothesis will remain unwarranted (unsupported by the data) and thus decrease the reliability of

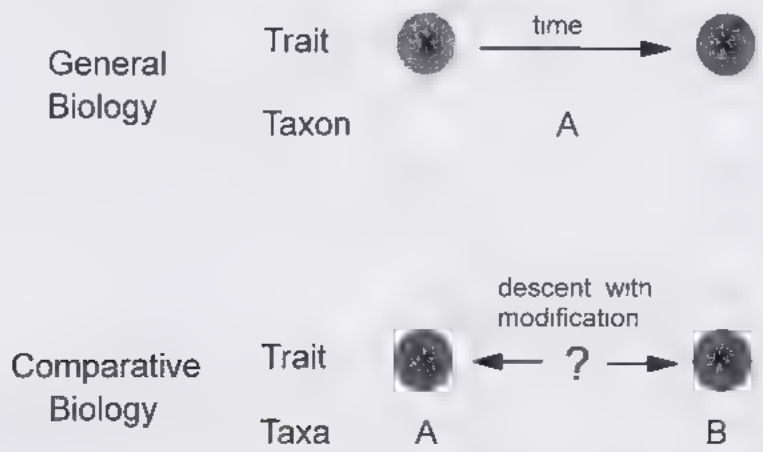

FIG 1 General biology deals with comparisons of different states of a trait "X" (X1 and X2) in a same taxon "A" at two different moments Comparative biology deals with comparisons of different states of a trait "X" (X1 and X2) in two different taxa "A" and "B" In comparative biology, one relies on an assumption of descent, which will remains hypothetical ultimately (here quoted with a question mark)

the results A usual argument for adding hypotheses that we called here "unwarranted" is to make analogy with previous case studies, in the way "it is well-known that evolution proceeds in the way " For example, "it is well-known that transversions are more frequent than transitions" This kind of argument seems to us clearly inappropriate in science in the absence of directly supporting evidence.

Taking into account the principle of independence There is another reason to decrease the number of *ad hoc* hypotheses To test evolutionary processes with phylogenetic patterns, it is

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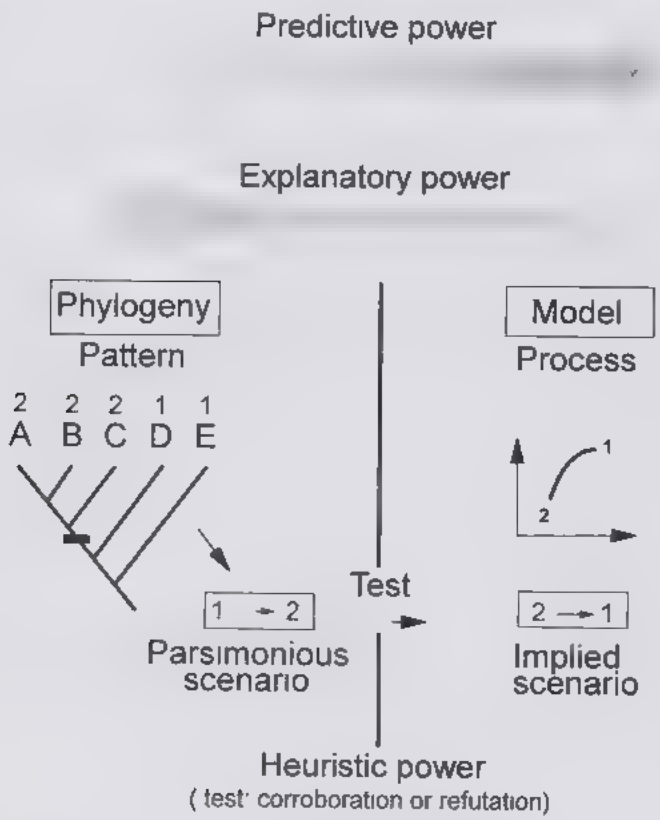

FIG 2 — The phylogenetic test of evolutionary scenarios compares two independent issues a pattern issued from a phylogenetic analysis (maximizing explanatory power) and a pattern issued from a model of evolutionary process (maximizing predictive power) The test itself has a maximal heuristic power, whether it provides a refutation or a corroboration as a result

necessary to follow the principle of independence (DELEPORTE, 1993, GRANDCOLAS *et al.*, 1994) One should not test hypotheses of evolutionary processes with phylogenetic patterns which would have been inferred using these same hypotheses The more *ad hoc* hypotheses used to infer phylogenetic patterns, the less validly evolutionary processes can be tested, *i.e.* tested with truly independent evidence.

The testing power of phylogenetic tests is inversely related to the number of *ad hoc* hypotheses made for reconstructing phylogenetic patterns Using a lesser number of *ad hoc* hypotheses, one could test and refute a higher ratio of evolutionary processes with a higher reliability This explicit principle is reminiscent of the earlier characterization of cladistics during the discussions among the different taxonomic schools HENNIG (1950) himself already distinguished phylogenetic systematics from evolutionary systematics on the basis of the use of fewer *a priori* assumptions, as quoted by DUPUIS (1984).

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Lessons from the evolutionary processes

The plea concerning this particular minimization of *ad hoc* hypotheses does not concern studies in general biology and especially in population biology These kinds of biological studies mainly deal with processes rather than patterns, and they study them in a diachronic way but in the *same* taxa the progress of a process can be observed along the time and the different states of a trait during a process may be put directly into relation without making too many hypotheses (Fig 1) Along the time, several parameters can also be monitored to study their influence on the process In this way, comparing a trait in the same species (or even in the same population of the same species) at different moments allows control of most influential parameters The comparison of two different states of the trait under study at two different moments does not necessarily increase the number of uncontrolled parameters This consequently does not decrease the number of degrees of freedom for these comparisons, as opposed to studies of comparative biology which compare different states of a trait in distinct taxa differing by many other characters

Population biology can thus develop fairly directly testable models Models formalize the relationships between several parameters on the basis of previous population studies Models make predictions which can be validated by further observations on populations The empirical validation of models is thus possible using complementary observations carried out at different moments on the same phenomenon (LEVINS, 1966, MICHALAKIS *et al.*, 1997, this volume) In general biology, predictions of models can be checked directly, while this is impossible for the same hypotheses in comparative biology Many models in general biology are predictive regarding evolutionary processes in populations and are considered only secondarily as predictive in different situations, at a macroevolutionary level and in different taxa These models acquire by extrapolation an heuristic value in comparative biology because their predictions can be addressed secondarily at a macroevolutionary level The validity of models at this level can no longer be assessed empirically because the observations are no longer repeatable in the same taxa It has been sometimes argued that validation may be possible however, using antagonistic models with opposite predictions (LEMEN & FREEMAN, 1989, MICHALAKIS *et al.*, 1997, this volume) But an identical prediction can be produced by several different models and thus cannot be validated solely by refutation of an opposite prediction generated by an antagonistic model (DUNBAR, 1989)

An evolutionary model at macroevolutionary level can only be validated by a comparison with the independent patterns which can be collected using phylogenetic analysis This is an important methodological justification of the usefulness of phylogenetic tests of evolutionary scenarios

Phylogenies versus models: explanatory power versus *predictive power*

Both approaches, phylogenetic analysis and process modeling, are obviously valuable for different reasons and they are complementary There is an opportunity to compare the models of processes in general biology and the phylogenetic patterns in comparative biology In this comparison, the patterns are testing the processes because patterns minimize *ad hoc* hypotheses at a macroevolutionary level while the models are *ad hoc* constructions at this level (Fig 2) Analyses of patterns and processes have contrasting powers (Figs 2, 3) Phylogenetic patterns have a high explanatory power (FARRIS, 1979, 1983), because available data are explained by

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themselves without any *ad hoc* additional hypothesis (Figs 2-3) Models of processes have a high predictive power, because they are designed to make predictions (Figs 2-3) The comparison of these two contrasted analyses has a higher heuristic power than each separate analysis (Fig 2) because conclusions obtained when maximizing explanatory power are compared with conclusions obtained when maximizing predictive power

	PHYLOGENY	EVOLUTIONARY MODEL
Concern	Pattern	Process
Object	Clade	Population / Clade
Power	Explanatory	Predictive
Level	Unique	Statistic
Reliability	Robustness	Validation
Pre-requisites	Descent with modification	Additional Hypotheses

FIG 3 — Contrasted characteristics of phylogeny and model, including respective concern, object, power, level, reliability and pre-requisites

With respect to these principles, parsimony is not used as a particular model of evolution but as a logic for reasoning using as few *ad hoc* hypotheses as possible (FARRIS, 1983) This point has particularly been misunderstood (*e.g.* PAGEL & HARVEY, 1989, PAGEL, 1994) and has been a blind alley in discussions for several decades as noticed by RIEPPEL (1988) and EGGLETON & VANE-WRIGHT (1994b) Parsimony must be used as a logical principle and it has inevitable consequences concerning the reconstruction of evolution However, any other method would be less valuable, because of the use of more *ad hoc* and unwarranted hypotheses Parsimony in data analysis for phylogeny reconstruction is like democracy in the popular joke "the worst system, but nobody has ever found a better one" Assertions such as "in this case, parsimony does not work" are soundless because one does not know how evolution has proceeded in a given case and one cannot propose a model to mitigate parsimony use which is free of additional and costly assumptions

It is sometimes asserted that phylogeny has also a predictive power (RIEPPEL, 1988, SYSTEMATICS AGENDA 2000, 1994), because it supplies parsimonious hypotheses of character states when one state is unknown within part of an ingroup This assertion is misleading because it confounds the causation and the effect of parsimony use Parsimony is used to provide hypotheses of phylogenetic patterns, even though some character states are unknown in some

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taxa, because a phylogenetic explanation is needed even with incomplete data But parsimony is primarily not used for predicting the value of missing data, such as unknown character states Used in this exclusive way, parsimony would be nothing else than a model, and a poor one, of phylogenetic inertia through extrapolation of character states present in the sister taxa The use of the term "predictive" should be restricted to modeling, it is misleading in the case of phylogenetic analysis and was probably mistaken for "heuristic", "informative", or better-conceived "explanatory".

TEST LIMITATIONS

Limitations can be intrinsic or extrinsic to the methodology of tests Some intrinsic limitations have been emphasized in recent criticisms and are the product of unwarranted predictions by particular models of evolution As these models cannot be validated, these hypotheses of limitations are not testable and are refuted in a first step Other intrinsic limitations deal with the very nature of cladistic phylogenetic hypotheses and should be taken into account A first limitation is related to the robustness of phylogenetic trees on which phylogenetic tests are based Many authors have stressed that phylogenetic trees are not necessarily correct and that studies based on phylogenies should consider carefully this point (*e.g.* EGGLETON & VANE-WRIGHT, 1994c) Although this point must be obviously a matter of concern, it could not justify rejection of phylogenetic tests based on phylogenetic trees which have been *correctly* assessed even according to only one set of data (either morpho-anatomical, or behavioral, or molecular, etc) As in any scientific study, a reasonable amount of evidence must be taken into consideration, even if additional evidence can possibly change the results in the future, provided that these results are refutable (QUIN & DUNHAM, 1983) It could be far less hazardous to use phylogenies even if they are young hypotheses still not much discussed in the literature than to use many *ad hoc* hypotheses to test evolutionary hypotheses Cladistic phylogenies and related phylogenetic tests – even based on limited evidence – can be refuted contrary to *ad hoc* hypotheses of macroevolution By the way, a further examination of the problem of tree robustness may be found in this volume (WENZEL, 1997)

A second intrinsic limitation deals with the absence of temporal scales when dealing with cladistics Minimizing unwarranted hypotheses such as "evolutionary clocks" precludes any possible absolute dating in cladistics (except minimal age estimates using fossils, which is evidence independent of cladistics *per se*) This is particularly detrimental to the comparisons between clades for testing hypotheses of niche displacement, coevolution, etc Conversely, studies which do not use this principle increase the burden of hypotheses For instance, the validity of the conclusions of OWENS & BENNET (1995) relies on their hypothesis of an evolutionary clock in bird clades, a hypothesis less than reliable (CRACRAFT, 1992, MINDELL, 1992, O'HARA, 1991).

Most other limitations stay far beyond the tests and are related to the general and statistical significance of the addition of the results of several tests (Fig 4) They are extrinsic to the tests but will undoubtedly become an important matter of concern when many phylogenetic tests are achieved in the future The addition of their results will allow generalizations (GRANDE, 1994), provided that tests are carried out without sampling bias These possible biases will be discussed in a second step

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Model-based criticisms

Recently, several authors have criticized phylogenetic tests, considering that parsimonious reconstructions do not work under the assumptions of particular evolutionary models (LEROI *et al.*, 1994, FRUMHOFF & REEVE, 1994, GRETHER, 1995, SCHLUTER, 1995)

A first criticism was based on a misunderstanding of phylogenetic tests According to LEROI *et al.* (1994), pattern and process would be confused in phylogenetic tests and the pattern would not be sufficient in itself to prove the existence of a corresponding process (for example, polarity testing for the adaptive value of a trait) But, many phylogeneticists do not make the assumption of an obligatory and reciprocal relationship between a kind of pattern and a kind of process (CARPENTER, 1989, CODDINGTON, 1990, GRANDCOLAS *et al.*, 1994) This point has been clearly explained by CODDINGTON (1990) who showed that phylogenetic tests of evolutionary scenarios contrast two patterns, one from the phylogeny and one implied by evolutionary process (the scenario) In this way, the phylogenetic pattern is not taken as a direct indication of the presence of a process but tests for its lack versus its *possible* presence The presence of this pattern in phylogeny is only a corroboration of the hypothesis of process A corroboration is always weaker than a refutation (BERNARD, 1865, POPPER, 1959), it cannot be taken as a proof and thus it is necessary to substantiate the hypothesis of process by additional

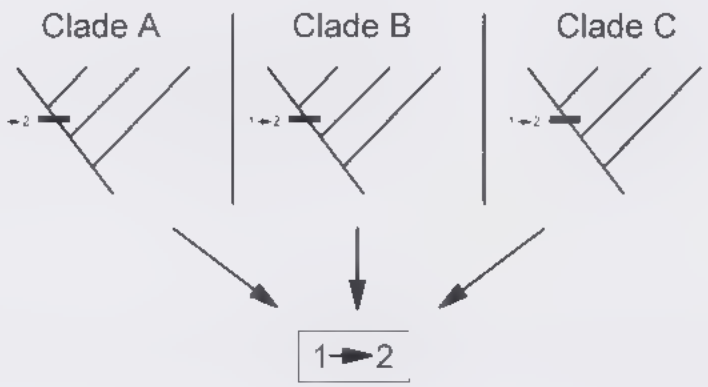

FIG 4 The generalization of a pattern (1 → 2) by the addition of phylogenetic analyses of three independent clades A, B and C This generalized parsimonious pattern must be compared to the underlying scenario of an evolutionary model

population studies For example, character polarity may corroborate an hypothesis of adaptation but cannot prove directly the adaptive value of this character The possible strong inference issuing from a phylogenetic test comes in fact from the observation of a phylogenetic pattern incompatible with the expected pattern, thus constituting a refutation of the tested process More precisely, it constitutes a refutation of the idea that the process would have existed and played a major role in orienting macroevolution in the considered clade The process is refuted by the phylogenetic pattern and not the contrary because it comprises much more unwarranted hypotheses at the macroevolutionary scale than the phylogenetic pattern It is always possible to imagine that the process existed and left no traces behind, but this is not a testable and scientific proposition

Source MNHN, Paris

A second criticism deals with the possible genetic linkage between several traits (FRUMHOFF & REEVE, 1994, LEROI *et al.*, 1994, GRETHER, 1995) According to this criticism, a strong genetic link could better explain the changes of certain characters than their own adaptive value This criticism is related to the misunderstanding commented upon above Still, if the phylogenetic pattern of a trait is incompatible with the pattern implied by a hypothetical process concerning this trait, there is refutation of the process hypothesis, whatever any possible role of genetic linkage As previously mentioned, if there is corroboration, there is still additional work to be achieved on populations before conclusion This additional work should include genetic studies of linkage (see also MORAND, 1997, this volume)

A third criticism, addressed more widely, concerns some general assumptions of evolutionary models Under specific evolutionary models dealing with rates or likelihoods of transitions and speciations, FRUMHOFF & REEVE (1994) and SCHLUTER (1995) imagined how phylogenetic tests could become inefficient in reconstituting past events This sort of model-based assumptions are easily testable in populations but are unwarranted at a macroevolutionary scale, *a priori* to any phylogenetic reconstruction (see CARPENTER, 1997, this volume, and SCHULTZ *et al.*, 1996 for arguing against the model of FRUMHOFF & REEVE, 1994) Even if some patterns constructed with cladistics are biased because of some particular modes of evolution, there is *a priori* no other means to reconstruct them The addition of the burden of any particular model would only make results less reliable because one can never substantiate this particular model concerning a past evolutionary phenomenon (analogy is not adequate in this respect to build a particular model)

These three kinds of criticisms either are based on a misunderstanding of the procedure of phylogenetic tests or do not follow a primary principle of comparative biology, that is to minimize unwarranted hypotheses

Actual limitations: beyond the individual tests

Particular as well as general hypotheses can be tested using phylogenetic patterns When dealing with general hypotheses, and to assess more strongly the conclusions, the phylogeny of several monophyletic groups may be studied to perform as many tests Monophyletic groups may be considered as having evolved independently if they are not directly related (not sister-groups, or one group not included in another) This assumption is only statistical as even if only a few symplesiomorphic characters are shared, they can possibly determine evolutionary processes in two clades which were hypothesized to be independent Consequently, if several tests bearing on different and independent groups provide the same results (refutation or corroboration of the hypothesis), the hypothesis is tested *by analogy* more strongly and generally In this way, a kind of statistical significance may be assessed using the addition of several phylogenetic independent tests (Fig 4) Such independent tests are not often possible today because of lack of available phylogenies The opportunities of carrying out phylogenetic tests are still scarce This should not preclude anticipating the future statistical pitfalls and the biases which could occur, but should incite to the realization of much more phylogenetic analyses

Delineation of the trait under study Depending on this delineation, the phylogenetic pattern may vary Trait delineation comprises the definition of the trait itself, the definition of its

Source MNHN, Paris

states and the establishment of primary homology A trait may be used in phylogenetic tests either as a character for building the tree, or as an attribute optimized afterwards on the tree Considering the trait either as a character or as an attribute depends on the primary homology of the trait (DE PINNA, 1991, GRANDCOLAS *et al.*, 1994), also named topographical correspondence by RIEPPEL (1988) The establishment of primary homology is often neglected although it is a

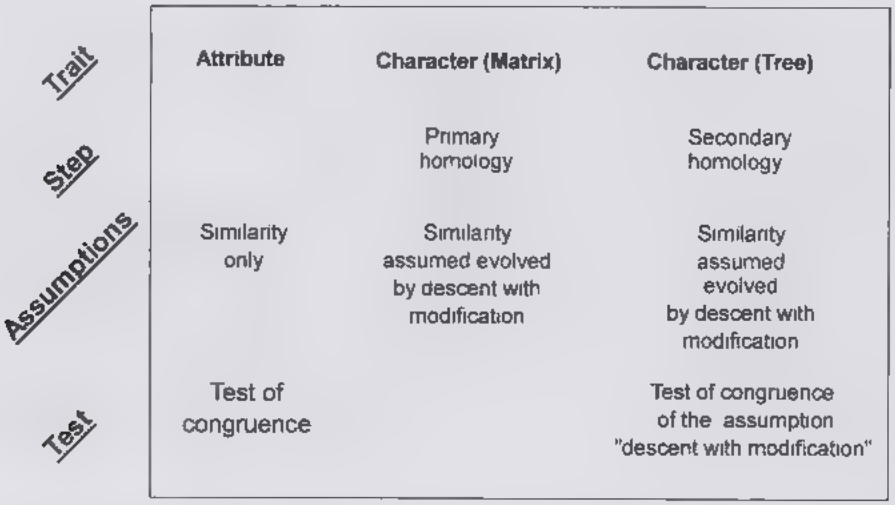

FIG 5 – The different operations applied during phylogenetic analysis to traits being attribute or character in a matrix, or character in a tree The attribute satisfies only to a statement of similarity, but not to a statement of homology, it is submitted to a test of congruence The character is firstly assessed primary homologous on the basis of its similarity and on the basis of an assumption of descent with modification it is secondly assessed secondarily homologous on the basis of a test of congruence of the assumption of primary homology

critical step in phylogenetic analysis (GRANDCOLAS, 1993, GRANDCOLAS *et al.*, 1994) The primary homology of a trait is arbitrarily assessed by using statements of similarity which themselves rely mainly on the heritability and the delineation of this trait (Fig 5) For example, traits such as geographical distributions may not be said to be strictly homologous because they are not heritable *sensu stricto* (DUPUIS, 1984) Also, macroecological traits such as "benthic" cannot be said homologous because they are defined at a too large scale (MICKEVICH & WELLER, 1991) and thus poorly defined Most disagreements concerning primary homology come from the definition of primary homology itself For example, all broadly similar traits could to be said to be primarily homologous (DEPORTE, 1993), even if they are not used to build a tree, because they are similar and coded as such when mapped on the cladogram afterwards This concept is however equivocal, in that it does not take into account the fact that these so-called homologous traits are not used as characters for building the tree, as all presumed *a priori* homologous traits should be with respect to the principle of total evidence (KLUGE, 1989) According to GRANDCOLAS *et al.* (1994), only similar traits which are used for building the tree should be said primarily homologous, they should be said to be only similar when optimized on the tree and when this mapping is the only way to assess their homology In other words, primarily homologous traits – characters – are by definition similar traits which are postulated *a priori* to

Source MNHN, Paris

be acquired by descent with modification and not to be homoplastic (Fig 5) Conversely, attributes are similar but are not *a priori* postulated acquired by descent with modification (Fig 5), and this is why one does not treat them as characters supporting phylogeny construction (but see in this volume· CARPENTER, 1997 for another distinction between characters and non-characters, or WENZEL, 1997 for arguing in favor of all traits taken as characters)

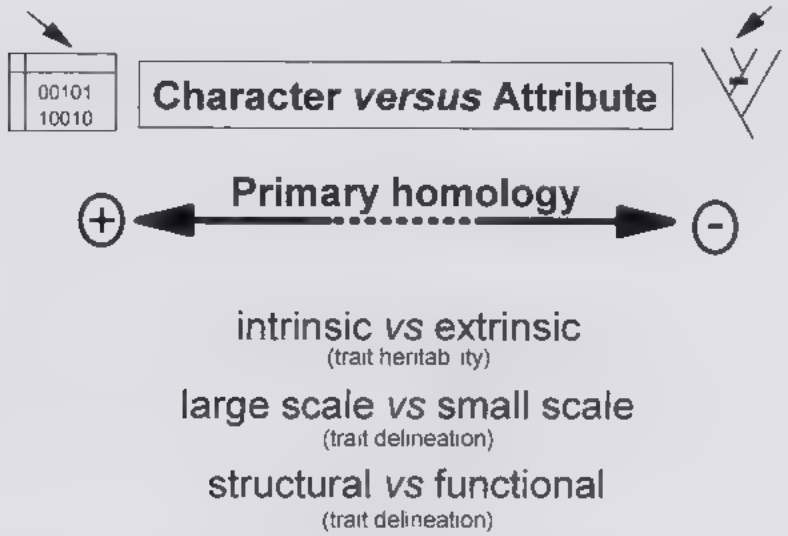

FIG 6 The distinction between character and attribute by the mean of a primary homology statement This statement concerning a trait is based on the perception of its nature intrinsic versus extrinsic (heritability), structural versus functional (delineation) and the scale large or small at which it has been defined previously (delineation)

Increasing both the accuracy of the definition and the number of states improves primary homology because the criteria of homology may be more easily applied to the trait (Fig 6) In this way, more available phylogenetic information existing in the traits is used A trait the primary homology of which is assessed can be used to build the tree and is thus submitted to an internal test of congruence with other characters (Fig 5) Increasing both the accuracy of the definition and the number of states optimizes in turn the secondary homology of the trait When the primary homology of the trait has not been assessed, this trait can be optimized (as an attribute) on the tree to discover its phylogenetic pattern This pattern can be more precise if the definition of both the trait and its states are accurate

Concerning the problem of character delineation and especially the "character versus attribute" alternative, one should be aware that primary homologies should not be indirectly assessed Unfortunately, homologies of behavioral or ecological traits are often based not really on direct examination of the criteria of homology but on indirect considerations For instance, the homology of a behavioral trait is often assessed according to its neural or its anatomical correlates If homology of the neural scheme or anatomical structures are assessed, we would better use neural schemes or anatomy as characters Also, homology is often assessed using circular reasoning, especially in broadly similar traits behavioral trait is observed in two taxa

Source MNHN Paris

known to be closely related, and so it is considered homologous, provided that they are related This is obviously circular Homology is not independently assessed for the ethological trait itself but by using a model of phylogenetic inertia Determining the homology of behavioral traits is however possible using the classical criteria of homology, but actually applied to behavior itself Most problems of plasticity and variability which are often said to prevent assessing behavior homology must be solved by appropriate ethological studies (WENZEL, 1992)

Selection of the ingroup This term refers here to the selection of a group of taxa supposedly monophyletic, without any contingencies related to the sampling of taxa The ingroups are generally studied for *a priori* reasons of suitability for specific phylogenetic tests of characters Ingroups are often studied also according to some constraints of feasibility are the taxa well known, have their phylogeny or at least their characters been preliminarily studied? A phylogenetic test deals with the evolution of one or several traits from an ancestral state toward derived state(s), possibly including reversals, this means that the group on which the test is carried out comprises taxa showing at least two states for each trait Also, the groups under study are generally relatively small, still because of constraints of feasibility Phylogenetic studies of larger groups are rarely carried out because many more character state occurrences must be documented according to the increased number of terminal taxa Ingroups are consequently most often relatively *small* in size and *diverse* with respect to the trait under study Consequently, patterns inferred from these phylogenies will be submitted statistically to scale effects Comparing the results of several phylogenetic tests carried out on different clades could lead to a bias which, in turn, could prevent a statistical estimate of the general prevalence of a pattern and to assess the validity of the model corresponding to this pattern For example, if someone wants to study the evolution of flying kinematics and behavior in insects, he would probably focus on Diptera, as this is the order which is currently very diverse and well-known in this respect But he would not analyze the whole order of Diptera because to examine hundreds of taxa in this group will overwhelm his capacity to carry out phylogenetic studies within a few years Thus, he would select a few groups which are *smaller*, which have been already partly studied, and which are *diverse* with respect to flying behavior Selected groups should necessarily be diverse (character diversity), otherwise no comparative study may be carried out for want of different states of traits to be compared

As they are statistically smaller and more diverse than if they were truly taken randomly in the tree of life, ingroups may present a non-random selection of patterns which are used to test evolutionary processes In our example, our Dipterist would have certainly not selected very large taxa with very few variation in flying behavior (*e.g.* a monophyletic tribe comprising 500 species, of which 499 have a first kind of flight and only one another kind) These groups would be excluded from the analyses Afterwards, generalizations based on these studies would not take into account patterns which could be more frequent in large and homogeneous groups This non-random selection may be expected to be particularly biased Indeed, the diversity of a given character should statistically increase with the size of a group Thus, choosing small and diverse groups excludes most of groups present in a given part of the tree of life, those which are larger and moderately diverse, and those which are of the same size and which are not diverse

Source MNHN Paris

The patterns and the relevant tested processes (Figs 7-8, see also GRANDCOLAS *et al.*, 1994) are listed below with the possible bias induced by the choice of the ingroup The biases are mentioned below provided that all things are equal otherwise in the ingroup and in the tree of life, except the ingroup size and the diversity of the character under study in this ingroup These biases may be expected statistically only (*i.e.* for a large number of ingroups), it is obvious that a unique and particular group may not conform to the statistical expectation

Polarity (testing for adaptation, Fig 7) size and diversity of the ingroup may or may not have particular scale effects regarding this pattern/process Polarity cannot be expected to have a particular value in a small and diverse group and only depends on the distribution of character' states on the taxa and on the structure of the phylogenetic tree

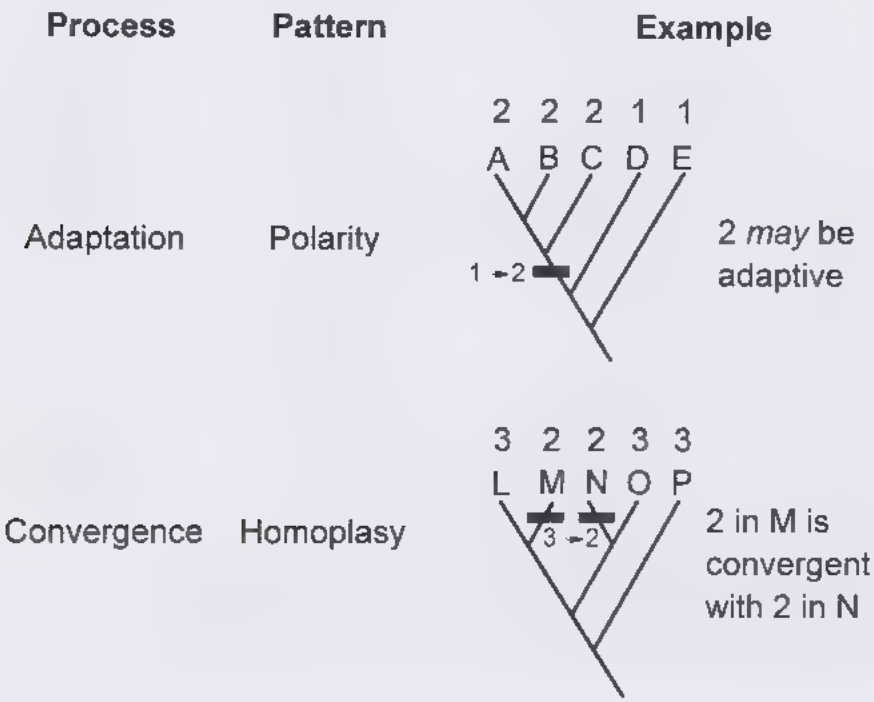

FIG 7 — Two patterns relevant to the phylogenetic test of two processes (see GRANDCOLAS *et al* , 1994 for more details). From left to right, the process to be tested, the pattern to be searched for testing, an example of phylogenetic test with its issue

Homoplasy (testing for convergence, Fig 7) small and diverse ingroups may present statistically less homoplastic patterns because of the decrease of the number of subordinated nodes after a change in character state The bias concerning this pattern is only related to the size of the ingroup. small ingroups do not allow to document as many reversals as could be expected because small ingroups have statistically fewer nodes If there is a change of states of a character at a given node, there is simply more cases with no existing subordinated nodes which could permit to document another subsequent change of state such as a reversal

Source MNHN Paris

– Time lag (testing for coadaptation-exaptation, Fig. 8) when testing for coadaptation or exaptation, (relative) time lag between the changes of two traits or between a trait and its function are searched for in phylogenies Using smaller and diverse groups, there is a lower number of nodes where changes can take place. This can bias the correlation studies between two traits after the change of a first trait, subsequent changes could take place in fewer places Consequently, a smaller number of changes will necessarily be observed This will bias the

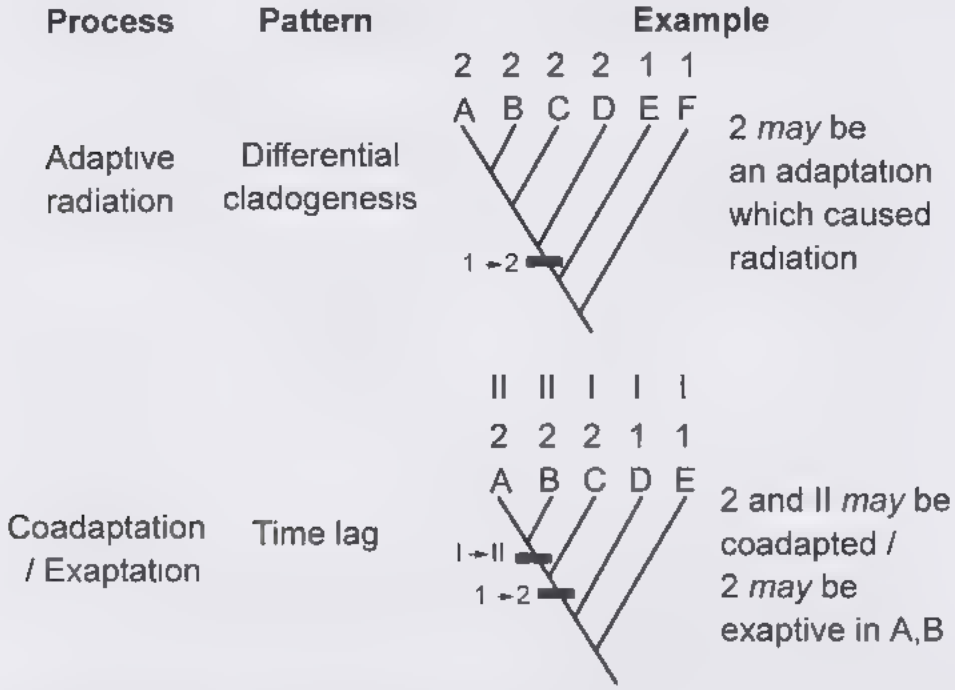

FIG 8 — Two other patterns relevant to the phylogenetic test of two other processes (see GRANDCOLAS *et al* , 1994 for more details). From left to right, the process to be tested the pattern to be searched for testing, an example of phylogenetic test with its issue

frequency of observed time lags and will provide us with fewer corroborations of coadaptation-exaptation This statement does not refer to a probabilistic approach for testing coadaptation-exaptation, such as that presented by MADDISON (1994) for challenging the views of SILLÉN-TULLBERG (1988) Probabilistic approaches deal with events occurring within the clades while our statement concerns the statistical meaning of (in)congruent results obtained from several clades

Differential cladogenesis (testing for radiation, Fig 8) small ingroups with a high number of evolutionary changes cannot show relatively differential cladogenesis concerning the trait under study. Important differential cladogenesis can exist by definition only in very large ingroups because they imply a high number of taxa in the subgroup where occurred the most important cladogenesis This can prevent to test for the importance of adaptive radiation which is

Source MNHN Paris

the process corresponding to the phylogenetic pattern of differential cladogenesis (GUYER & SLOWINSKI, 1991) This can prevent conversely testing for the role of evolutionary stasis, because the chosen small ingroups with a high number of evolutionary changes may not show evolutionary stasis

Smaller ingroups are also *statistically* and *relatively* more recent groups, compared to larger ingroups, provided that both are taken in the same inclusive monophyletic group Depending on the stability of evolutionary rates, this could lead to study only the relatively more recent evolutionary events This is detrimental to the tests of evolutionary hypotheses which are linked to particular climatic or geological periods (but we can note that using too large a group could also lead to irrelevant correlations between a relatively old phylogenetic pattern and much more recent geological or climatic events) It should be kept in mind that the relation between ingroup size and age is not absolute but statistical There also exist a few small and relatively old groups among all possible ingroups taken in the same inclusive monophyletic group (*e.g.* the so-called "relict taxa")

The last bias, but not the least, is related to the relevance of the ingroup for testing a particular evolutionary model The phylogenetic test is designed to refute or to corroborate the prediction of an evolutionary model taking into account a number *N* of factors The model could not be tested correctly when only *(N 1)* factors are considered in the phylogenetic test This situation would occur if *(N 1)* factors are represented as apomorphies in the ingroup and if the *N th* factor is represented by a symplesiomorphy of the ingroup This factor/plesiomorphy could make either trivial or extremely rare the pattern corroborating the model and could thus bias strongly the test toward corroboration or refutation A recent example may be found in studies of Hymenoptera, where reversals of sociality were documented in Halictidae using phylogeny PACKER *et al* (1994) interestingly questioned why so many sociality reversals occur, while no appearances were documented Together with other reasons, the phylogenetic inertia may have been quite important in biasing the tests In Hymenoptera, most theories of social evolution put forward the role of brood care for favoring sociality Higher-level phylogenetic analysis shows that brood care (the *N th* variable) is ancestral to Halictidae and this could bias the study toward a minimization of appearance events Only studies at a much wider phylogenetic scale could adequately document appearances of sociality, for instance succeeding to the appearance of brood care and not preceding it Another example deals with the origin of complex reproductive behaviors in cockroaches These behaviors - ovoviviparity and viviparity evolved following the appearance of "deposition of ootheca after sclerotization", which is apomorphic in cockroaches, relative to mantids and termites (GRANDCOLAS, 1996) If the females did not keep their ootheca after sclerotization, they could not have evolved toward subsequent retraction and nutrition of oothecae in a brood sac (ovoviviparity and viviparity) Anyone who would like to study subsequent evolution of reproductive behavior in a particular group of cockroaches should not forget that the character "deposition of ootheca after sclerotization", plesiomorphic at this level, is still influential (ROTH, 1989)

CONCLUSION

Comparative biology is still a young and growing research field, as was phylogenetics when HENNIG (1965) published one of his last methodological accounts Following the development of

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phylogenetic methodology, it is now necessary to elaborate a cohesive methodology which can take into account the possible interrelations of phylogenetic patterns with evolutionary processes (and relevant models) This is generally made through the phylogenetic test of patterns which are expected under some process hypotheses

As a contribution to this methodology, three rules are proposed which could improve phylogenetic analysis both intrinsically and extrinsically These improvements should increase the phylogenetic test power and decrease the test limitations

First, the burden of hypotheses in phylogenetic analysis should be reduced by decreasing the number of unwarranted hypotheses (with parsimony use) Comparative biology proceeds using hypotheses only Adding unwarranted extra-hypotheses is detrimental to the reliability of the results

Second, the independence of phylogenetic patterns relative to process hypotheses should be enhanced the same way, by decreasing the number of *ad hoc* hypotheses used to infer them Particularly, to test an hypothesis of process, one should not use patterns inferred using this same process hypothesis.

Third, statistical bias during the generalization of the tests should be minimized When several similar tests are carried out on different ingroups, their results may be compared to generalize them The possible peculiarities of ingroups should be taken into account to minimize the possible bias in the generalization

The first two rules deal with a general problem encountered in many research fields of evolutionary biology Minimal hypotheses (sometimes named null hypotheses or null models, *e.g.* PATTERSON, 1994) are wanted in comparative studies as well as in population studies of adaptation (GOULD & LEWONTIN, 1979) or in studies of biotic interactions (QUINN & DUNHAM, 1983) These minimal hypotheses are needed to check the validity of the *ad hoc* hypotheses used to reconstruct the past Both a lack of minimal hypotheses or an abuse of *ad hoc* hypotheses will make the results flawed or unreliable It is stressed that comparative studies should take this principle into account, for consideration paid to previous methodological analyses in evolutionary biology We must not reinvent the wheel in comparative biology, disregarding methodological advances in phylogenetics or in evolutionary biology

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Comparative Analyses of Continuous Data: the Need to Be Phylogenetically Correct

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ABSTRACT

In this paper I focus on the problem of non incorporating phylogenetic information when doing a comparative analysis. A review of the theory on this subject shows that not incorporating the phylogenetic information inflates the degree of freedom and can increase the risk of type I and type II errors of statistic tests done on cross species data (non phylogenetically controlled). The phylogenetic independent contrasts method (FELSENSTEIN 1985) has been developed to resolve the problem of non-independence of data (i.e., traits measured across different species) in comparative studies. After a presentation of the assumptions of this method, I provide one example on parasite species richness of mammals which shows the errors that lead to false conclusions. For example, a non phylogenetic approach (cross species comparisons) would lead to the conclusion that parasite diversity is linked to host body size, whereas a phylogenetic independent comparison shows no relationship between host body size and parasite richness. A non phylogenetic approach would thus lead us to reject the null hypothesis when it is false (Type I error). One assumption underlining the independent contrasts method is the random walk model (Brownian motion) which is used as a null hypothesis. Many traits that are considered in comparative studies are unlikely to be well described by a simple Brownian motion process. I propose to use MANTEL tests to detect evolutionary trends in comparative analyses. I performed a simulation that shows the efficiency of MANTEL tests for detecting evolutionary trends and for measuring phylogenetic effects. MANTEL tests could be one answer to the critical comments made on the independent contrasts method.

RÉSUMÉ

Analyse comparative des donnees continues : la necessite d'être « phylogenetiquement correct »

Dans ce travail, je m'interesse aux problemes lies a la non prise en compte des informations phylogenetiques quant on réalise une analyse comparative. Une revue de la théorie concernant ce sujet montre que de ne pas incorporer les informations phylogenetiques augmente le degre de liberté et accroît les risques d'erreur de type I et de type II des tests statistiques effectues sur les donnees non controlees pour la phylogenie. La methode des contrastes independants (FELSENSTEIN 1985) a été développee pour resoudre le probleme de la non-independance des donnees (les traits mesures chez les differents taxons, dans les études comparatives). Apres une presentation des hypotheses de cette methode, je donne un exemple concernant les richesses parasitaires des mammifères terrestres qui montre les erreurs conduisant a des conclusions erronees. Ainsi, une approche non phylogenetique aurait conduit a la conclusion que la diversite parasitaire est liee a la taille de l'hôte, alors que la methode des contrastes independants montre l'absence de relation entre ces deux variables. Une approche non phylogenetique peut conduire a rejeter l'hypothese nulle alors qu'elle est vraie (erreur de type I). Une des hypotheses de la methode des contrastes independants est le modele de marche aleatoire (mouvement brownien). De nombreux traits, pris en compte dans les analyses comparatives, ne sont pas bien decrit par le modele de mouvement brownien. Je propose d'utiliser

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Source: MNHN, Paris

les tests de Mantel pour detecter les tendances evolutives dans les analyses comparatives. J'ai conduit une simulation qui montre l'efficacite des tests de Mantel pour detecter les tendances evolutives et mesurer les effets phylogenetiques. Les tests de Mantel peuvent être une des reponses aux critiques effectuees sur la methode des contrastes independants.

INTRODUCTION

There are two ways for analyzing evolutionary processes. The first one, the population approach, focuses on micro-evolutionary processes and tries to find adaptation at work, *i.e.* the evolution of a specific character under natural selection or sexual selection. The second one, the comparative method, tries to identify adaptation by studying the evolution of a specific character, in different lineages, supposed to be driven by the same selection pressures. The development of cladistic analyses has challenged the definition of adaptation. For example, CODDINGTON (1988) has defined an adaptation as an apomorphic function promoted by natural selection. I will concentrate on the second approach.

First of all, we have to distinguish the differences between phylogenetic effects from phylogenetic constraints. DERRICKSON & RICKLEFS (1988) have drawn the attention on the fact that numerous biologists do not make the difference between phylogenetic effects and phylogenetic constraints. According to these authors, the phylogenetic effects are only the expression of the tendency of related species to be similar because they share a common history. They defined a phylogenetic constraint as the effect of history onto the changes in diversification of a given clade or as the differences in evolutionary interactions between a phenotype and its environment. However, as emphasized by MCKITRICK (1993) such definition refers more to the results than to the causes of a constraint. MCKITRICK (1993) suggested that a constraint highlights the absence of a given character or the lack of an expected evolution. She proposed the following definition where a phylogenetic constraint is "any result or component of the phylogenetic history of a lineage that prevents and anticipated course of evolution in that lineage". The lack of viviparity among birds is an example of phylogenetic constraint.

Very early, people have recognized several pitfalls linked with cross-species comparisons. It has been recognized that taxonomic relationships greatly influence the correlation between the analyzed traits (STEARNS, 1992). Interspecific comparison is a very common approach in ecology (as well as in other branches of biology). Many recent studies, and even recent textbooks, in ecology or evolutionary biology continue to ignore these statistical pitfalls and persevere to ignore the importance of the phylogeny and the history of organisms.

Some evolutionary biologists use parsimony methods for inferring the evolution of a particular character. GARLAND & ARNOLD (1994) argued that the application of parsimony analyses can be justified only on methodological grounds but do not refer to any model of evolution (but see SOBER, 1994 for the use of parsimony in evolutionary biology). FELSENSTEIN (1988) challenged the view that reconstructing phylogenies is a statistical problem and implies an explicit model of evolution. People interested in the evolution of discrete characters mostly use parsimony analyses whereas those dealing with continuous characters use independent comparative methods (but see PAGEL, 1994).

It is not my aim to compare these two very different methods (parsimony *versus* independent comparative method) for the analysis of adaptation. Rather, I focus deliberately on the statistical approach in order (1) to convince evolutionary ecologists about the need to control for phylogeny when comparing different species, (2) to draw the attention of

Source: MNHN, Paris

phylogeneticists to models (and statistics) that underline every methods, (3) to propose MANTEL tests as a method to detect evolutionary trend

HOW TO REVEAL PHYLOGENETIC EFFECTS? A FIRST APPROACH

FISHER & CHAPMAN (1993) tried to answer to this question by analyzing the dispersal mechanisms of plant fruit The objectives of their study were to examine the degree to which plants have evolved predictable, disperser-specific syndromes and to determine the consequences of using different taxa as sampling units when analyzing comparative data to test for the existence of dispersal syndromes These authors recognized that using species as independent sample units implies that the analyzed character (fruit morphology) should have evolved independently in any clade, which is not self-evident Furthermore, an analysis based on species will dramatically inflate the number of events In the absence of a fully resolved phylogeny, FISHER & CHAPMAN (1993) proposed to use genera as sample units The hypothesis is that if the apparition of a given trait is the result of convergent evolution then this correlation should always be found when using genera as sample units Because the correlation was lost using genera as sample units, FISHER & CHAPMAN (1993) concluded that a study based at the species level is not unbiased This example highlights two major problems First, the use of taxonomic information is arbitrarily and, second, the use of species as independent points may lead to false conclusion

WHY USING PHYLOGENETIC INFORMATION IN COMPARATIVE ANALYSES?

Three pitfalls should be avoided in comparative analyses:

(1) not incorporating phylogenetic information may inflate the degrees of freedom,

(2) high risk of rejecting H_0 when it is true (Type I error),

(3) high risk of accepting H_0 when it is false (type II error).

Not incorporating phylogenetic information implies that we make the assumption of a true case of multiway speciation events ("hard polytomies", MADDISON, 1989), which refers to a star phylogeny However, most phylogenies are dichotomous even if some parts are unresolved (soft phylogeny) Imagine the case of 5 species, a star phylogeny gives (5-2 3) degrees of freedom while a dichotomous phylogeny gives (5-3 2) degrees of freedom or less (GARLAND & ARNOLD, 1994)

Figure 1, redrawn from GITTLEMAN & LUH (1992), shows the problem of phylogenetic relations Suppose a known phylogeny with 2 genera and 6 species By plotting trait variations and ignoring phylogenetic pattern we might find a relationship whereas it is erroneous (type I error false rejection of H_0) Conversely, we might reject a relationship (type II error false acceptation of H_0) which actually exists

I will give below an example showing both statistical errors.

THE INDEPENDENT CONTRASTS METHOD

The phylogenetic independent contrasts method (FELSENSTEIN, 1985, MARTINS & GARLAND, 1991, PAGEL, 1992, GARLAND, 1992) has been developed to resolve the problem of non-independence of data (*i e.* traits measured across different species) in comparative studies FELSENSTEIN (1985) suggested a procedure for calculating comparisons between pairs of taxa at each bifurcation in a known phylogeny (Fig. 2)

Source MNHN, Paris

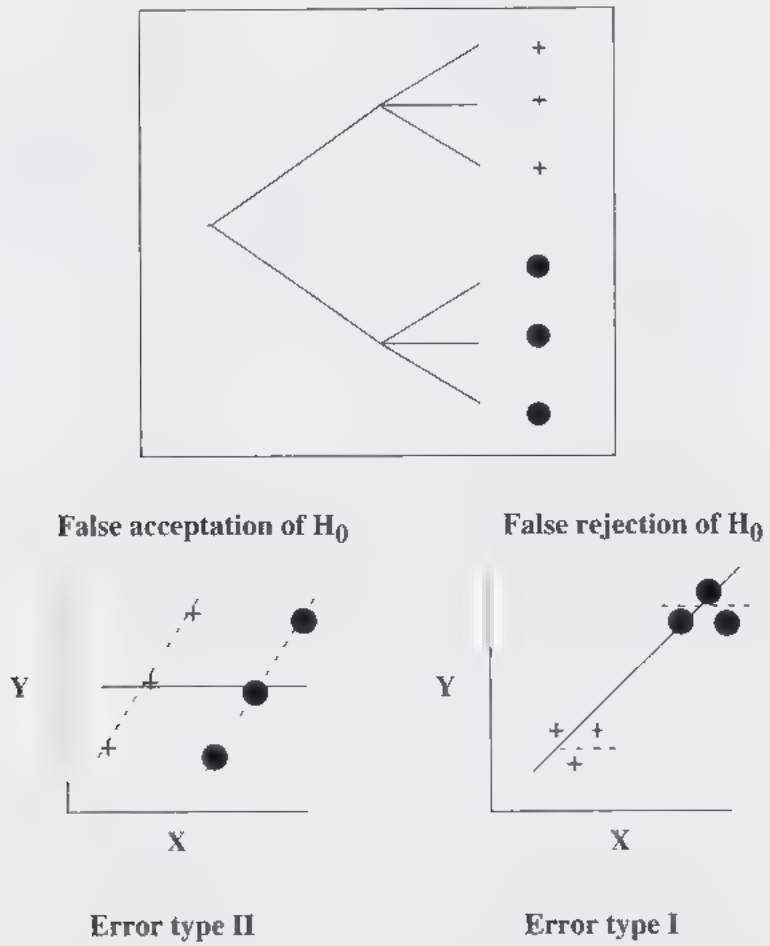
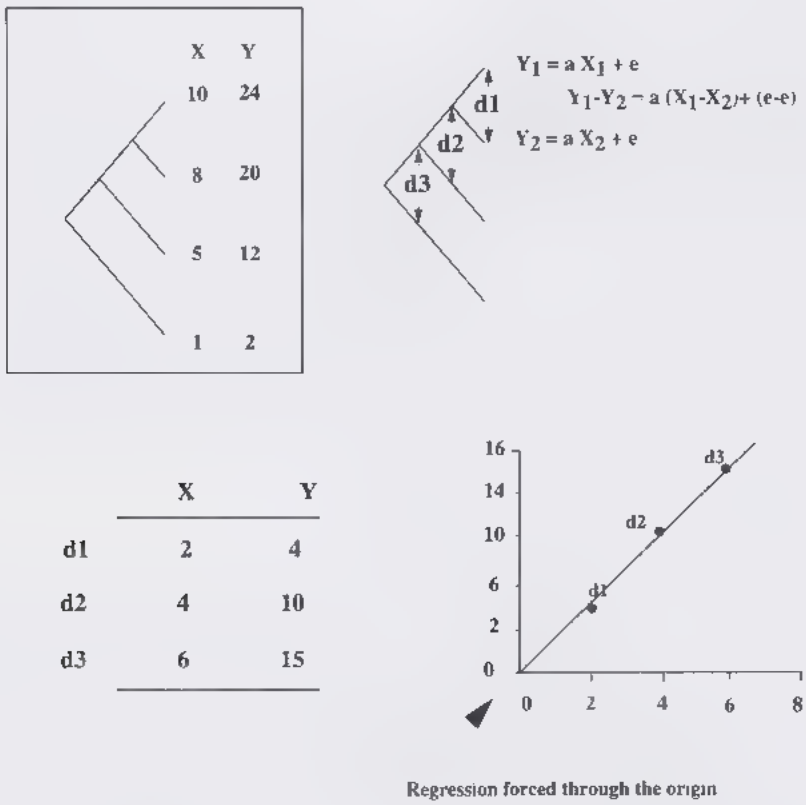


FIG 1 — Ignoring phylogenetic relationships may lead to erroneous conclusions A Type I error (false acceptation of the null hypothesis) occurs when rejecting the extant correlations (dashed lines) whereas a Type II error (false rejection of the null hypothesis, occurs when claiming correlation (solid line, when its actually false (dashed lines) The illustration is after GITTLEMAN & LUH (1992)

In a phylogenetic tree, the independent events (on which an analysis can be performed) correspond to the nodes that give rise to daughter branches For each branch of a node, values for a given variable are obtained by averaging the values of its own daughter branches Then the difference for each variable between the two daughter branches of each node is calculated In the calculation of contrasts, the direction of subtraction is arbitrary Multiple nodes can be treated in a way that gives a single contrast (PURVIS & GARLAND, 1993). Pairs of sister branches that diverged a long time ago are likely to give greater contrasts than pairs of sister branches that diverged recently It is thus necessary to standardize each contrast through division by its standard deviation where the standard deviation of a contrast is the square root of the sum of its branch lengths (GARLAND *et al.*, 1992) In the absence of information on branch length, one can assume each branch length to be equal to unity Another method is proposed by GRAFEN (1989) for assigning arbitrary lengths In this method the age of a node is assigned as the number of

Source MNHN, Paris

daughter groups descended from that node minus one Nevertheless, GARLAND *et al* (1992) showed that using arbitrary or real branch lengths often leads to similar results In order to check that contrasts are properly standardized it is suggested to perform a regression of the absolute values of standardized contrasts versus their standard deviations In case of positive relationship it is necessary to transform branch lengths before computing standard deviations (GARLAND *et al.*, 1992) All correlations between contrasts are forced through the origin (Fig 2)



	X	Y
d1	2	4
d2	4	10
d3	6	15

FIG 2 The independent contrasts method The illustration is after GITTLEMAN & LUH (1992) and PURVIS & RAMBAUT (1995)

The three main assumptions of independent contrasts are

(1) a correct topology,

(2) branch lengths measured in units of expected variance of character evolution,

(3) a Brownian motion model of character evolution or random walk model (FELSENSTEIN, 1985, 1988)

Under a Brownian motion model of evolution, a change in the mean phenotype is expected to be non-directional and to occur at a constant rate This rate can be described in terms of the relation between the variance among species phenotypes and time as

$$V_b = \beta t + \varepsilon$$

Source MNHN, Paris

As pointed out by MARTINS (1994), many traits that are considered in comparative studies are thought to have been the subject to the action of natural or sexual selection. Thus, these traits are unlikely to be well described by a simple Brownian motion process. The performances of the independent contrasts under different models of character evolution have been tested (see MARTINS & GARLAND, 1991, MARTINS, 1994, BJÖRKLUND, 1994). Simulation studies indicate that the independent contrasts method produces acceptable error rates. Moreover, the independent contrasts method produces less error rates than other phylogenetic correction methods, like nested ANOVA or phylogenetical autocorrelation (MARTINS & GARLAND, 1991, PURVIS *et al.*, 1994, DIAZ-URIARTE & GARLAND, 1996).

Three statistical assumptions must be tested when working with a real data set (GARLAND *et al.*, 1992, PURVIS & RAMBAUT, 1995).

(1) the random walk model can be tested by regressing the absolute values of the standardized contrasts against the estimated nodal values,

(2) homogeneity of variances can be tested by regressing the absolute values of the standardized contrasts against the height or ages of the corresponding nodes,

(3) and ANOVA can be used to test for heterogeneity of variances amongst multiple node values.

However, one problem with the independent contrasts method is the accurate estimation of the ancestral values at ancestral nodes (PAGEL, 1992). The method of averaging values can introduce several biases. Excluding ancestral nodes from the analysis is one way to test if the relationship remains identical with actual species (PAGEL, 1992).

PARASITE RICHNESS OF MAMMALS AS EXAMPLE

I compiled data on nematodes recovered from 66 species of terrestrial mammals. These data were collected from several sources based on a survey of 90 studies published over the last 30 years. Comparative analyses of parasite species richness should avoid 2 pitfalls: sample size (GREGORY, 1990, WALTHER *et al.*, 1995) and phylogenetic confounding effects (HARVEY, 1996). As GREGORY (1990) and WALTHER *et al.* (1995) pointed out, investigations on parasite species richness must take into account differential sampling effort. Differential sampling effort is a consequence of both the researcher's sampling procedure and of the geographical range of the hosts, and both may affect host and researcher encounters, and thus directly influences the observed number of parasite species.

The need to take the phylogeny into account is related to the coevolution between hosts and parasites. Hence, host phylogeny may be important in determining the richness of a parasite community (HOLMES & PRICE, 1980, BROOKS & MCLENNAN, 1991). Furthermore, cross-species comparisons performed using species values as independent data points may be confounded by the phylogenetic relationship of the analyzed species (FELSENSTEIN, 1985, HARVEY & PAGEL, 1991, MARTINS & GARLAND, 1991). For example, a correlation between host body size and parasite species richness may arise because a group of related and same-sized hosts have a high parasite species richness because of their common phylogenetic origin and not because of common ecological forces. Closely related species tend to be similar. Therefore, species values cannot be treated as statistically independent points (HARVEY & PAGEL, 1991).

I based the analysis on the working phylogeny of mammals (Fig. 3) proposed by POULIN

Source: MNHN, Paris

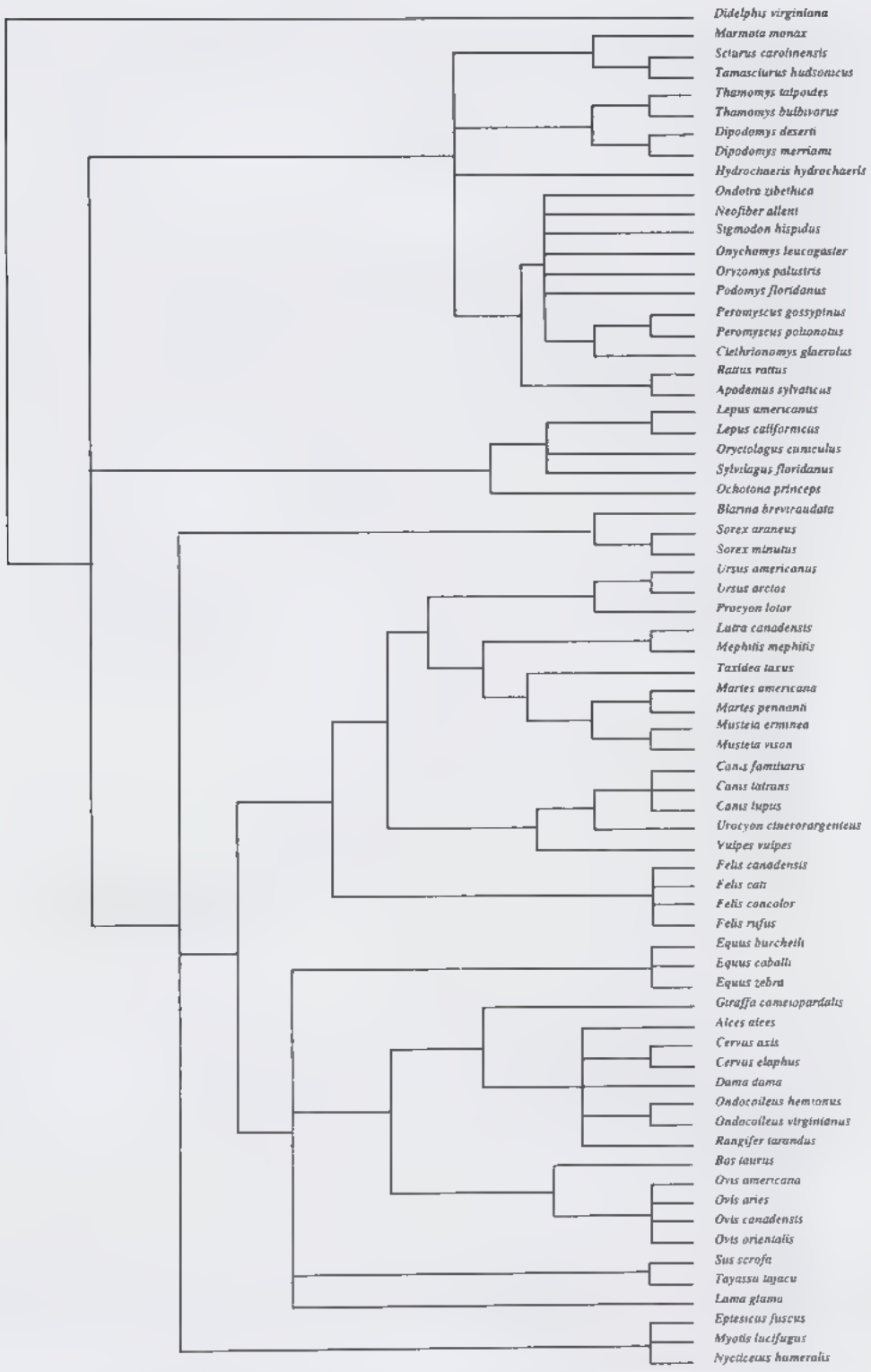


FIG 3 Phylogeny of mammals used in the analysis (this phylogeny redrawn from POLLY 1995 is based on various sources molecular and morphological data)

Source MNHN, Paris

(1995) I used the C A I C program (PURVIS & RAMBAUT, 1995) Data on parasite species richness and host body lengths were logarithmically transformed (HARVEY, 1982) Because parasite species richness can correlate with sampling effort, both variables were controlled for host sample size before the analyses All correlations between contrasts were forced through the origin (GARLAND *et al.*, 1992)

Parasite richness and host body size

Cross species analysis and phylogenetic independent method gave rise to different results (Fig 4) A non phylogenetic approach (cross species comparisons) leads to the conclusion that parasite diversity is linked to host body size However, a phylogenetic independent comparison of contrasts analysis showed no relationship between host body size and parasite richness A non phylogenetic approach would lead us to accept the null hypothesis when it is false (Type I error) My results support those of POULIN (1995) who also did not find any relationship between mammal body size and parasite species richness when correcting for host phylogeny

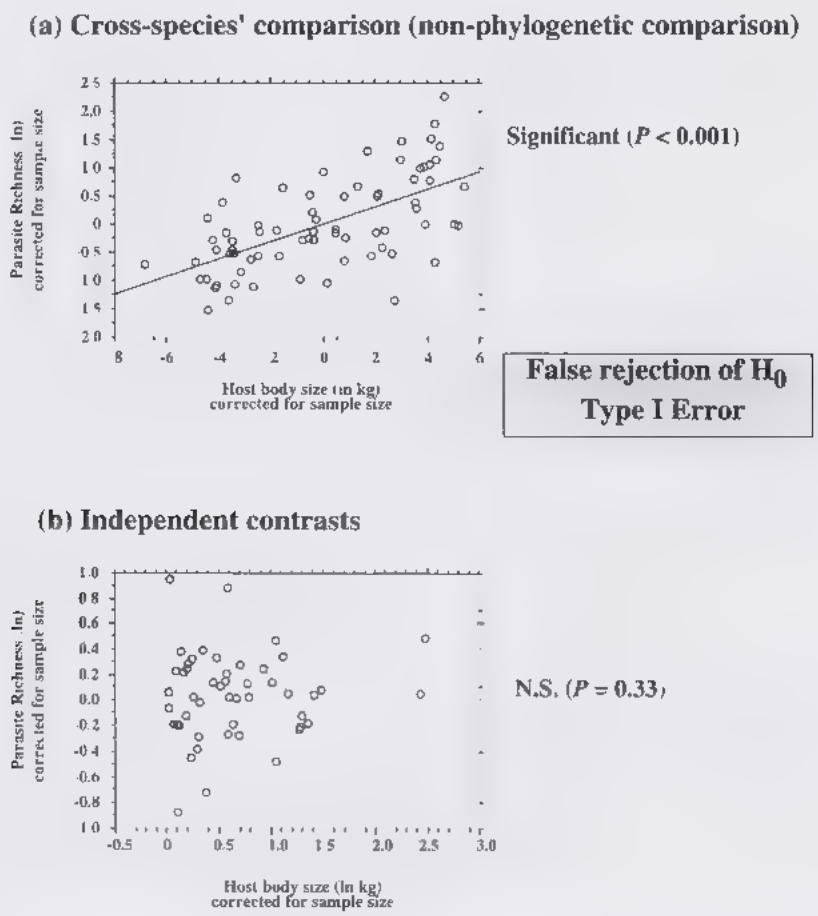


FIG 4 A significant relationship between host body size and parasite diversity (nematodes) is found when using a non-phylogenetic approach whereas it is false as detected by the independent contrasts method Parasite species richness is controlled for sampling effort

Source MNHN Paris

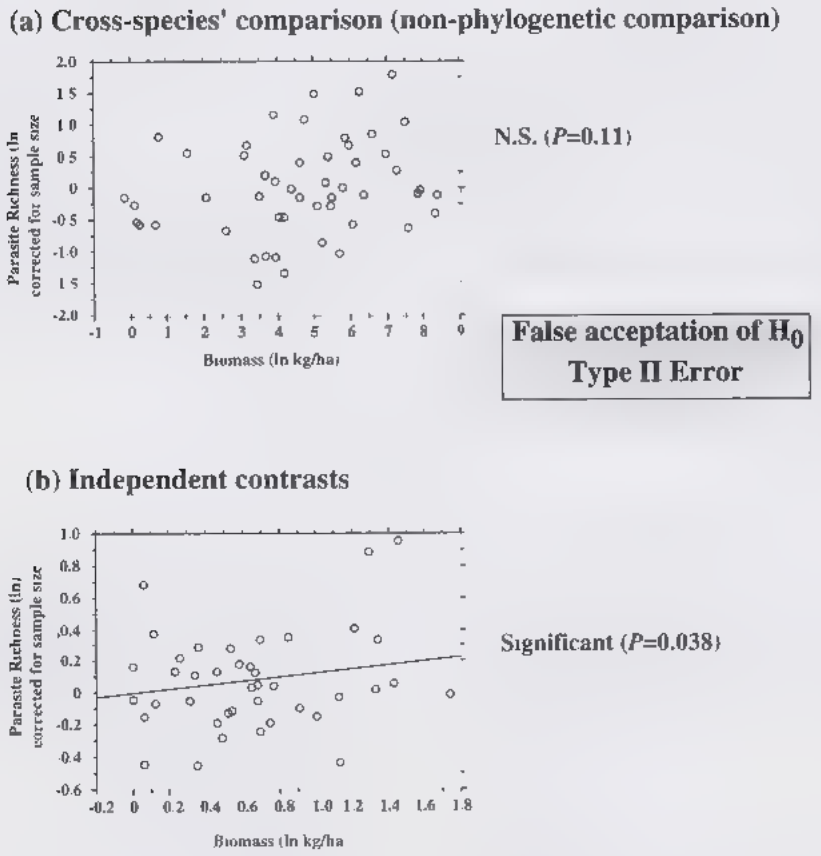


FIG 5 — A lack of relationship between host biomass and parasite diversity (nematodes is found using a non-phylogenetic approach whereas the independent contrasts method detects a positive relationship

Parasite richness and host biomass

The results found by the two methods were also different While a non-phylogenetic approach did not detect any relationship between the two variables (Fig 5), the independent comparison allows to find a significant relationship between nematode diversity and host biomass Thus, a non phylogenetic approach will lead to accept the null hypothesis whereas the null hypothesis is wrong (Type II error)

DETECTING EVOLUTIONARY TRENDS AND THE USE OF MANTEL TESTS

Analyzing evolutionary trends was the topic of the essay of MCKINNEY (1990), who proposed time series analyses as a tool for detecting an evolutionary trend. For McKinney, trends are persistent statistical tendencies in some variables (such as morphological) in an evolutionary time span De facto, random walk (Brownian motion) is used as a null hypothesis MCSHEA (1994) argued that large-scale evolutionary trends may be passive or driven Whereas the passive trend may correspond to a Brownian motion of character evolution (random walk), the driven trend corresponds to a selection-driven system (MCSHEA, 1994)

Source MNHN, Paris

Both systems of evolution (passive or driven) yield to the conclusion that related species share the same characters due to their phylogenetic proximities However, in a passive system distant species can share the same characters because of the random evolution of characters (Brownian motion)

I performed a simulation study, to show that MANTEL tests cannot detect pure Brownian motion of character evolution (passive trends) but can detect driven evolutionary trends with acceptable error rates MANTEL tests have been used to quantify phylogenetic effects (TAYLOR & GOTELLI, 1994), and an extended version of this test has been proposed by LEGENDRE *et al.* (1995) However, the robustness of the MANTEL test in comparative analyses has not yet been evaluated

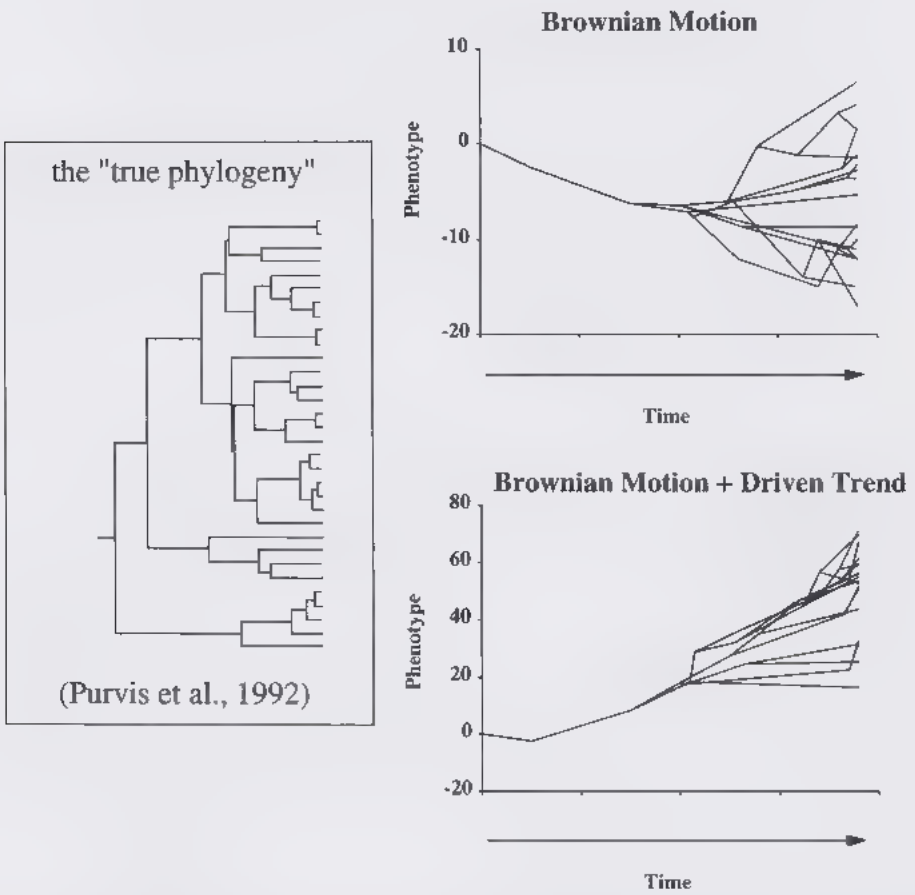


FIG 6 The "true phylogeny" used in the simulation study The changes in variance among species phenotypes with time are shown under a Brownian model of evolutionary change (with $\sigma^2 = 1$ throughout clade) and under a Brownian + a driven evolutionary trend

Source MNHN, Paris

Methodology and examples

Using a modified version of PURVIS *et al* 's methodology (1994) to take into account a driven trend, values of pairs of characters, Y and X, were generated for the 32 species along the phylogeny given in Fig 6 For each branch segment, the changes of values of these traits are given by

$$\Delta X = N(0,1)^* \sqrt{branch\ length} + pi(\beta)$$

$$\Delta Y = a.\Delta X + (1 - a)\, N(0,1)^* \sqrt{branch\ length}$$

where N(0,1) is a normal pseudo-random number of mean 0 and variance 1, a is the input correlation and pi(β) the probability of increase (see below) Each normal random number is multiplied by the square root of the branch length (following PURVIS *et al* , 1994) Starting from the root of the tree, where X = 0 and Y = 0, values at successive nodes i are computed as

$$X(i+1) = X(i) + \Delta X$$

$$Y(i+1) = Y(i) + \Delta Y$$

The values of X and Y for the species, located at the tip of the branches, were calculated by summing the changes along all branches of the phylogeny

In a passive system (pure Brownian motion), pi = 0 In a driven system, the value b (10 in my simulations) is added to ΔX according to a probability of increase pi (pi = 0 9, I used the same value as in MCSHEA, 1994) The passive system corresponds to the simulation method of PURVIS *et al.* (1994) whereas the driven system follows a similar methodology to that exemplified by MCSHEA (1994)

I calculated 1000 pairs of X variable with a = 0 and used them for detecting errors of

	Mantel tests	
	X and Phylogeny	Y and Phylogeny
Pure Brownian		
test of validy (Type I)	p>0.05	p>0.05
test of power (Type II)	p>0.05	p>0.05
Brownian + Driven trend		
test of validy (Type I)	p<0.05	p>0.05
test of power (Type II)	p<0.05	p>0.05

FIG 7 Mantel test method In Mantel tests, the X variable is transformed into distance matrix **X**, by computing the "distance" among values (absolute value of the difference) The phylogeny is represented by a matrix **P** of patristic distances among species Patristic distances are computed as the lengths of segments along the evolutionary tree that separate two species The regression of the individual values in the matrices yields the regression coefficients constructed by Monte Carlo simulation (MANLY, 1991) The significance (p) was determined by Monte Carlo simulation

Source MNHN, Paris

Type II. Similarly, I used a further set of 1000 pairs with a value for a 0 3 for detecting errors of Type I (I used the same value as PURVIS *et al.*, 1994).

In Mantel tests, the X and Y variables are transformed into distance matrices **X** and **Y**, by computing the "distance" among values (absolute value of the difference). The phylogeny is

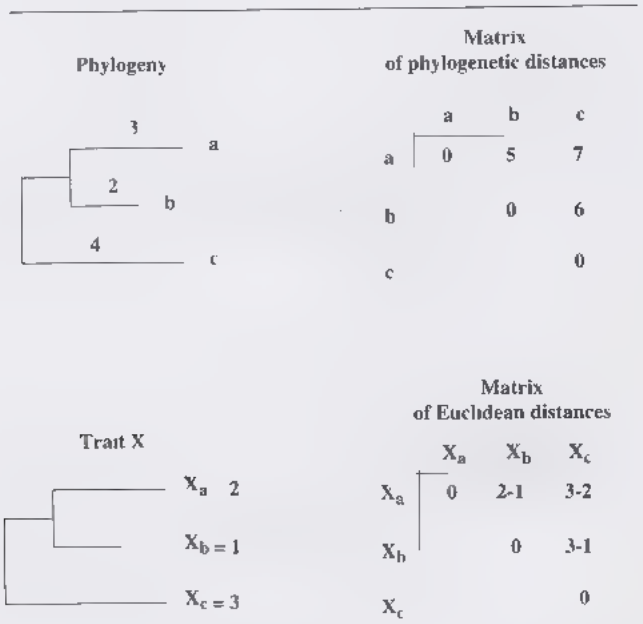


FIG 8 Results of the simulation study for a passive system (Brownian motion of character evolution) and a driven system (phylogenetic trend). Test of validity (detection of type I errors) is carried out using a fixed input correlation of a 0. Test of power (detection of type II) is performed using a fixed input correlation of a – 3. Mantel tests were done between variable X and the matrix of the phylogeny (999 permutations each for the MANTEL test).

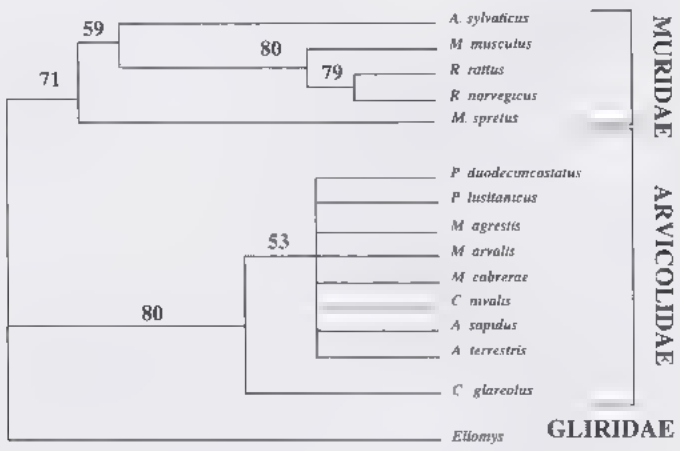


FIG 9 — Working phylogeny of rodents. Evolutionary divergences between rodents were obtained from various sources: paleontological records, morphological and molecular data (see FELIU *et al.*, 1997).

Source: MNHN, Paris

represented by a matrix **P** of patristic distances among species Patristic distances are computed as the lengths of segments along the evolutionary tree that separate two species (Fig. 7)

I implemented the Mantel test according to MANLY (1991) The regression of the individual values in the matrices yields the regression coefficients constructed by Monte Carlo simulation (SMOUSE *et al.*, 1986, MANLY, 1991) The significance (p) was determined by Monte Carlo simulation (999 replications) (LEGENDRE *et al.*, 1995)

According to my hypothesis, Mantel tests cannot detect a passive trend but can detect a driven trend based upon both validity (a ⁻ 0) and power tests (a 0 3) (Fig 8) The detection is found only for the X variable, which was the variable affected by the driven trend Based upon these results, it may be possible to detect a phylogenetic trend in comparative analyses This can be seen in the following real data sets the parasite species richness of Iberian rodents and the parasite species richness of African cyprinids Using data on parasites of rodents, collected over an eighteen year period on the Iberian peninsula, FELIU *et al.* (1997) investigated the determinants of parasite species richness in Iberian rodents More than 70 species of helminth parasites (nematodes, cestodes and digenes) were identified among fifteen species of rodents, for which a working phylogeny has been proposed (Fig 9) Parasites were classified into groups according to their host specificity Specificity corresponds to the number of infected host species by a given parasite species. the larger the host species number, the lower the specificity One explanation of parasite species richness is linked to host phylogeny A Mantel test shows that richness of specific parasites (corrected for host sample size according to WALTHER *et al.*, 1995)

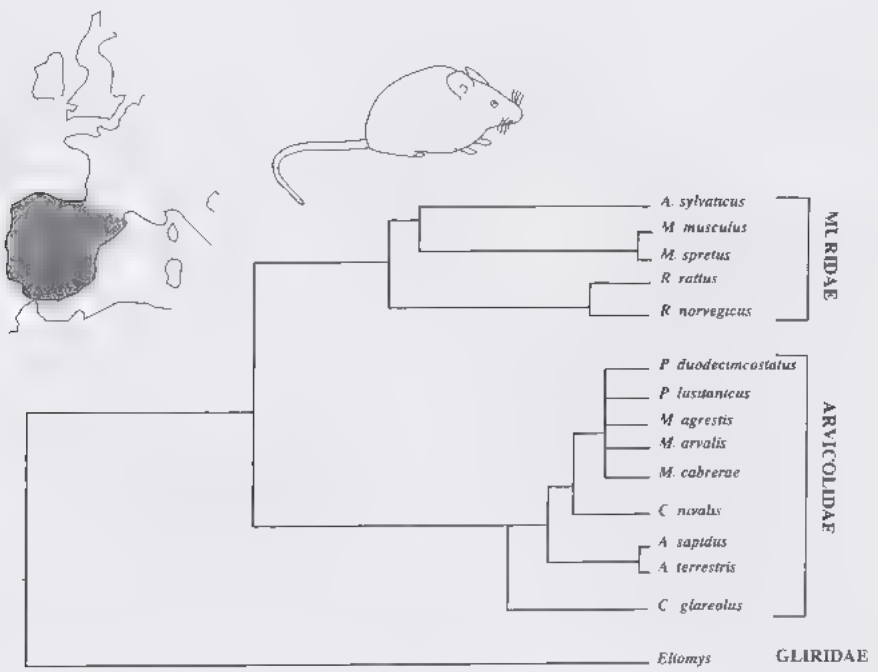


FIG 10 — Relationship between rodents using parasites as characters in a parsimonious construction tree (FELIU *et al.*, 1997 Specific parasite species are coded as characters values of bootstrap analysis are given on the figure, 100 replicates Note that major phylogenetic relationships are found

Source MNHN, Paris

is correlated with the phylogeny of their host ($p = 0.001$, $R = 0.66$) This pattern is clearly illustrated when using parasite species as characters for a tree reconstructing host relationships (Fig 10) The obtained consensus tree reflects the major phylogenetic divisions of the host group Thus, the detection of a phylogenetic trend, the increase of parasite species richness through the diversification of their hosts, is revealed by MANTEL tests and confirmed by tree reconstruction

GUÉGAN *et al.* (1992) investigated the richness of monogeneans (ectoparasites) of cyprinid fishes and found that host length is a major determinant of ectoparasite diversity More recently, GUÉGAN & MORAND (1996) have shown using the independent contrasts method that parasite species richness is correlated with changes in the level of host ploidy Because of the loss of explanatory power (percentage of variance) when using independent comparison, we may suggest that history of the host group can partially explain parasite species richness In this case, I used a MANTEL test (Fig 11) and found that phylogeny effectively explains a substantial amount of variance of species richness ($p < 0.001$, $R = 0.16$) In other words, this finding suggests that related species of hosts tend to have the same parasite species richness because most of the parasites have been inherited from their common ancestors

These two examples illustrate how Mantel tests can be applied in comparative analyses However, I would like to emphasize that the lack of detection of a phylogenetic correlation does

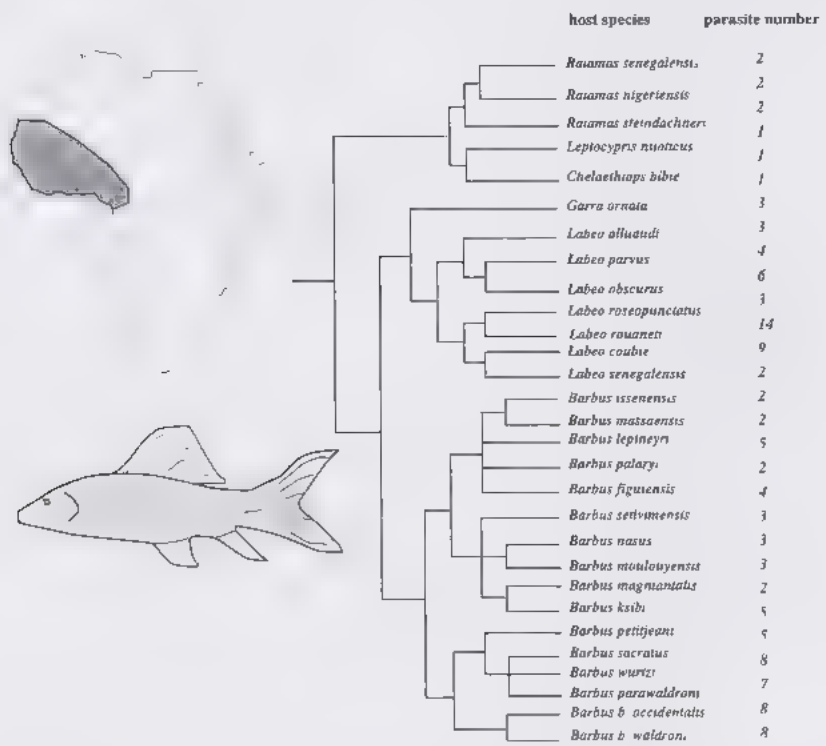


FIG 11 — Phylogeny of African cyprinid fish based on isoenzymes data (from GUÉGAN & MORAND, 1996) with number of parasite species

Source MNHN, Paris

not allow to the conclusion of the absence of phylogenetic effects The simulation studies clearly show that Mantel tests do not detect passive evolutionary trends (pure Brownian motion of character evolution) and that comparative studies should always use the independent contrasts method

SKEPTICISM ABOUT COMPARATIVE METHODS?

Before concluding, it is necessary to mention some problems concerning the use of comparative methods in evolutionary biology Two different criticisms have been put forward, one by LEROI *et al.* (1994) and the other one by WESTOBY *et al.* (1995a, 1995b).

LEROI *et al.* (1994) argued that comparative methods are "valuable for examining the evolutionary history of traits but they will often mislead in the study of adaptive processes" Their major concern is that we know very little on the evolutionary genetic mechanisms responsible for distributions of traits among species. They claimed that it is very difficult to justify any evolutionary scenario without evidence of historical selection forces and, more important, the genetic relations among traits Some of their arguments concern mainly the invocation of constraints in the explanation of either adaptation or phylogenetic conservatism However, the problem is more a problem of definition (what is a phylogenetic constraint) than a problem of method (the use of comparative method) A second set of arguments addresses the question of the evolution of continuous characters, the topic of this study Using the example of the scaling of brain and body size, described as a power function, they found at least two problems of the comparative method The first is that of confounding selection pressures I cannot see why this is a specific problem of the comparative method A correlation constitutes no proof whether the correlation is the result of the comparative method or any other methods The second criticism deals with "the confounding of the causal influence of selection with that of genetic correlations" This is a more serious critique but, again, the problem is more related to the causes and correlations than to methods Indeed, LEROI *et al.* (1994) concluded their study with the acknowledgment "that the methods of comparative biology and genetics might be usefully combined".

The second criticism came from WESTOBY *et al* (1995a, 1995b) Their concern was that a phylogenetic correction (i e phylogenetic analysis) is not a correction but rather a conceptual decision which gives priority to one interpretation over another In fact, they assumed that part of variation of a given trait is correlated with phylogeny and other part correlated with ecology However, their arguments refer to the notion of phylogenetic niche conservatism This process can be described as follows "the ancestor of a lineage possesses a constellation of traits, enabling it to succeed in a particular habitat and disturbance regime, through a particular life history and physiology The lineage will therefore leave most descendants in similar niches This niche conservatism in turn will tend to sustain a similar constellation of traits in descendants of the lineage (WESTOBY *et al.*, 1995a) HARVEY *et al.* (1995) gave a clear answer to that questions by emphasizing that the independent contrasts method does not remove phylogenetic effects but produces plots in which all the variation of the data set in one variable is graphed against all the variation in the other variable In this way, phylogenetic niche conservatism means that adaptations to different components of the niche will be correlated (HARVEY *et al.*, 1995), which is what the contrasts method has been designed to detect

Source MNHN, Paris

CONCLUSION

Within a multi-species study, species do not necessarily represent independent data points (KELLY & PURVIS, 1993, HARVEY, 1996). The recent debate involving WESTOBY *et al.* (1995a, 1995b) and HARVEY *et al.* (1995) highlighted some misinterpretations of comparative methods.

Comparative biologists have drawn attention to all the biases which could arise when the phylogenetic information are not taken into account (PAGEL & HARVEY, 1991, GARLAND *et al.*, 1992, MARTINS, 1995, HARVEY, 1996). Moreover, as emphasized by GARLAND & ARNOLD (1994), caution should be exerted to all comparisons involving only two species (there is no degree of freedom!).

In this study, I have provided one example on parasite species richness of mammals which showed these biases. Not incorporating phylogenetic information would have lead to false conclusions.

The independent contrasts method remains the best method to avoid the phylogenetic confounding effects (HARVEY, 1996, but see BJÖRKLUND, 1994, for a comparison of this method with character mapping by optimization on a cladogram). Even if the independent contrasts method assumes a model of character evolution (the Brownian motion model or any other models, see Martins, 1994), simulation studies showed that this method is very robust (low error rates). However, without a correct phylogeny of the studied organisms it is impossible to test evolutionary hypotheses. The main problem is the availability of a correct phylogeny. Recently LOSOS (1994) proposed to use computer simulations to generate a large sample of possible phylogenies in the absence of a correct topology and to calculate independent contrasts for each generated tree. LOSOS (1994) gave two rules of thumb. First, if all analyses give the same result (significant or not), then the result is independent of what the true phylogeny is. Second, if a substantial minority of phylogenies yield different results from the majority, then the outcome of the analysis will depend on the correct phylogeny.

There are some other methods in comparative analyses which solve the problem of non-independence (LYNCH, 1991), for example, the phylogenetic autocorrelation method (GITTLEMAN & KOT, 1990) or the permutation on distance matrices method (LEGENDRE *et al.*, 1995, MORAND, 1996, MORAND *et al.*, 1996). All these other methods have not been tested for their power in a wide range of character evolution (but see PURVIS *et al.*, 1994, MARTINS, 1995). I carried a simulation study showing the efficiency of Mantel tests for detecting evolutionary trends and for measuring the phylogenetic effect. I hope that Mantel tests will be an answer to the questions of WESTOBY *et al.* (1995). Mantel tests done on the data set (each variable against the phylogeny) will indicate if there is a trend in the changes of the values of each variable. We should remember that the lack of correlation may not lead to the conclusion of the independence of species. A correlation may indicate that the character does not evolve under a pure Brownian motion. The Mantel tests reveals a phylogenetic niche conservatism or, in the case of parasite diversity, a phylogenetic trend but they do not allow to avoid a phylogenetic independent analysis.

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Phylogenetic Tests of Evolutionary Scenarios: the Evolution of Flightlessness and Wing Polymorphism in Insects

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ABSTRACT

Secondary loss of the flight ability has occurred in nearly all winged orders of insects many times within most orders, and probably hundreds of times within the Hemiptera and Coleoptera Loss of flight may be an attribute of all individuals of a species, of only one sex (usually the female), or populations may be polymorphic, composed of both flying and flightless individuals, eventually with a seasonal variation in frequencies Since flightlessness is linked to a multitude of morphological, physiological, and ecological components that are of great evolutionary significance, flight loss and wing polymorphism in insects have received much attention in recent years The present paper focus on the role phylogenetic inference can play in clarifying evolutionary patterns of flightlessness in insects and possible causes of the loss of flight ability This approach is here exemplified using the orders of pterygote insects, the families of Hemiptera-Heteroptera (true bugs), the genera of the heteropteran family Gerridae (water striders) and the species of the water strider genera *Limnoporus*, *Aquarius*, and *Gerris* Cladograms can be used to track associations between flightlessness and other factors as well as the relative evolutionary success of flying and flightless sister groups However, phylogenetic inference applied to higher taxonomic levels presents many problems as exemplified by the orders of pterygote insects and the families of Hemiptera-Heteroptera In many cases obligatory loss of wings coincides with a significant change in way of life, *e g* ectoparasitism or marine habit, but it is rarely possible to tell which came first It is argued that phylogenetic inference are most effectively applied at lower taxonomic levels, in particular to monophyletic groups of species with varying expressions of wing polymorphism In species of northern temperate water striders (Heteroptera, Gerridae), phylogenetic inference shows definite associations between flight loss and durational stability of habitats Contrary to previous hypotheses the winged state is not the ancestral one Surprisingly the ancestors are inferred to be predominantly flightless or permanently dimorphic occupying relatively stable habitats Subsequent evolution of long-wingedness or seasonal dimorphism has allowed descendant taxa to colonize less stable habitats

RESUME

Tests phylogénetiques de scenarios évolutifs : l'évolution de la perte du vol et du polymorphisme alaire chez les Insectes

La perte secondaire de la capacité à voler a eu lieu dans presque tous les ordres ailés d'insectes, de nombreuses fois chez la plupart des ordres et probablement des centaines de fois chez les Hémiptères et les Coléoptères La perte du vol peut être un attribut de tous les individus d'une espèce ou de l'un des sexes (le plus souvent les femelles), ou bien encore les populations peuvent être polymorphes, c'est à dire composées d'individus capables ou incapables de voler, avec la possibilité d'une variation saisonnière de leur fréquence La perte du vol et le polymorphisme alaire chez les insectes ont été l'objet de beaucoup d'intérêt ces dernières années parce qu'ils sont liés à une multitude de composantes morphologiques, physiologiques, et écologiques qui sont d'une grande importance dans le domaine de l'évolution L'étude présente concerne le

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rôle de .nference phylogenetique dans .a clarification des sequences evolutives de la perte du vol chez les insectes et des causes possibles de cette perte Cette etude prend en compte les ordres d'insectes Pterygotes, les familles d'Hemipteres-Heteropteres (punaises), des genres d'Heteropteres Gerridae (patineurs), et des especes des genres de patineurs *Limnoporus*, *Aquarius* et *Gerris* Les cladogrammes peuvent être utilises pour retracer non seulement les associations entre la perte du vol et d autres facteurs mais aussi le succès evolutif relatif de groupes freres respectivement capables et incapables de voler Cependant, .'inference phylogenetique appliquee aux plus hauts niveaux taxonomiques pose de nombreux problèmes comme c est le cas des ordres d'insectes Ptérygotes et des famil.es d'Hemipteres-Heteropteres La perte totale des ailes coincide de nombreuses fois avec un changement important de mode de vie, *e g* , lectoparasitisme ou le mode de vie marin mais il est rarement possible de dire quel changement s'est fait en premier Il est donc propose que l'inference phylogénetique soit utilisee de maniere plus efficace et plus judicieuse a de plus bas niveaux taxonomiques, en particulier sur des groupes monophyletiques d especes montrant des types varies de polymorphisme alaire Dans les espèces de patineurs des zones temperees de l'hemisphere Nord (Heteropteres Gerridae), l'inference phylogenetique montre qu il existe une nette association entre « perte du vol » et « stabilite continue des habitats dans le temps » L etat aile n'est pas ancestral, ce qui est en contradiction avec les hypotheses anterieures De maniere inattendue, les ancêtres sont inferes avoir ete en majorite incapables de voler ou constamment dimorphiques, et avoir occupé des habitats relativement stables. L'evolution subsequente vers .a possession d'ailes longues et fonctionnelles ou vers un dimorphisme saisonnier a permis aux taxa descendants de coloniser des habitats moins stables.

INTRODUCTION

Flight capability is without doubt one of the primary innovations governing the evolutionary success of insects However, secondary loss of this capability has occurred in nearly all winged orders of insects, many times within most orders, and probably hundreds of times within the large orders of Hemiptera and Coleoptera The loss of flight may involve all kinds of modifications of wings and flight musculature Loss of flight may be an attribute of all individuals of a species, of only one sex (usually the female), or populations may be polymorphic, composed of both flying and flightless individuals and, eventually, with a seasonal variation in frequencies The largest variability in wing development is observed among water striders (Heteroptera, Gerridae) as illustrated in Fig 1

Flightlessness in insects is linked to a multitude of morphological, physiological, and ecological components that are of great evolutionary significance (*e.g.*, HARRISON, 1980, ROFF, 1986, 1990, WAGNER & LIEBHERR, 1992, SPENCE & ANDERSEN, 1994) The production and maintenance of the flight apparatus (wings, flight musculature, etc) is energetically expensive and bound to compete with other, physiologically equally demanding processes such as the production of oocytes ("oogenesis-flight" syndrome, JOHNSON, 1969) Therefore, one possible advantage of wing loss is that it allows a female insect to divert energy normally used in wing and wing muscle development to the production of more eggs This could increase the female's fitness more than the advantages associated with the ability to fly (ROFF, 1986, 1990, ROFF & FAIRBAIRN, 1991)

The most widely accepted explanation for loss of the flight ability in insects relates to environmental heterogeneity SOUTHWOOD (1962 172) predicted "that within a taxon one should find a higher level of migratory movement in those species associated with temporary habitats than in those species associated with more permanent ones" In his review of the evolution of flightlessness in insects, ROFF (1990) assembled considerable evidence indicating that flightlessness is strongly associated with habitat stability in all major groups of insects Habitats in which insects have a higher frequency of flightless forms than the average are woodlands, deserts, mountains, caves, ocean surfaces, termite and hymenopteran nests, and the body surfaces of homeothermic vertebrates (ectoparasites) Flightlessness has also been related to habitat

Source MNHN, Paris

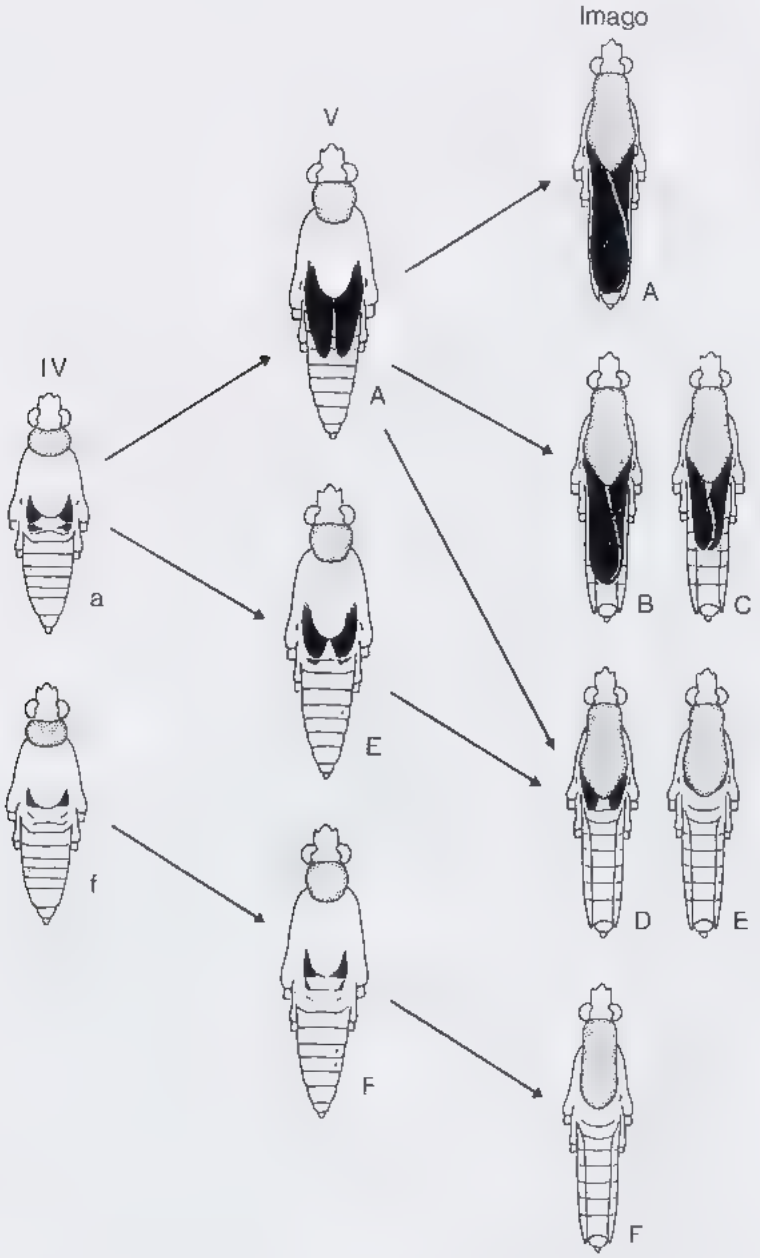

FIG 1 Wing polymorphism in water striders (Heteroptera, Gerridae and different ontogenetical pathways of wing development through fourth (IV) and fifth (V) instar nymphs and imago Nymphs and imagines shown without antennae and legs, not drawn to same scale Further discussion in text (Reproduced with modification from ANDERSEN, 1982)

isolation DARWIN's (1872) hypothesis that on oceanic islands a flightless morph would be more fit than a winged morph because it would be less likely to be accidentally blown or fly from the island was, however, questioned by ROFF (1990) who concluded that the proportion of flightless insects on islands was no higher than in continental areas

Source MNHN, Paris

Phylogenetic inference

The convergent evolution of flightless adult forms in taxonomically widely separate, primarily winged insect taxa suggests that the loss of flight ability and maintenance of wing polymorphism is a consequence of adaptation through natural selection as first proposed by DARWIN (1872) Nevertheless, previous discussions of the evolution of flightlessness in insects(*e.g.* HARRISON, 1980, ROFF, 1986, 1990, ROFF & FAIRBAIRN, 1991) have largely ignored the historical perspective in explaining patterns of flightlessness in insects The importance of phylogeny in comparative biological studies is now widely recognized (BROOKS & MCLENNAN, 1991, HARVEY & PAGEL, 1991, EGGLETON & VANE-WRIGHT, 1994, GRANDCOLAS *et al.*, 1994, MILLER & WENZEL, 1995) and this approach has recently been applied in studies of the evolution of flightlessness in insects (ANDERSEN, 1993a, ROFF, 1994).

What role can phylogenetic inference play in understanding the evolution of flightlessness in insects? First, reconstructed phylogenies (cladograms) can be used to track the evolutionary fate (*e.g.*, relative rates of speciation and extinction) of flightless and winged sister groups (ROFF, 1994) Second, cladograms may provide the basis for making phylogenetically more relevant comparisons among monophyletic groups (clades) Previously, correlation tests involving flight loss have assumed that every species of a clade represents an independent datum However, species sharing a trait (*e.g.*, wing loss) inherited from their most recent, common ancestor, do not yield statistically independent data Cladograms may also be used to predict which species require further study in order to resolve a particular problem Third and finally, by mapping different states of wing development upon a cladogram, ancestral states can be reconstructed, number of evolutionary transitions between states can be traced, and possible sequences of change can be inferred (ANDERSEN, 1993a) If two attributes are considered at the same time (*e.g.*, wing morphism and type of habitat), the relative position of state changes for the two attributes may be located If the flight ability was lost *after* a significant change in habitat, flightlessness may be an adaptation to the new habitat If flight loss preceded the change in habitat, flightlessness may be a prerequisite (exaptation) for the colonization of a new habitat It is important to remember, however, that in both cases flightlessness may be caused by other, presently unknown factors

Before using phylogenetic inference in comparative biological studies, the choice of taxonomic level should be seriously considered Ideally, the attribute(s) in question should vary much between, but only little within the groups selected as terminal taxa Within-taxon variability presents a major problem in most studies involving higher taxa (families, orders) which are more likely to show within-taxon variation than, *e.g.*, genera and species groups As a rule, the only solution to the problem of high within-taxon variability is to repeat the analysis with a more finely resolved phylogeny

MATERIAL AND METHODS

Phylogenies

The phylogenetic relationships between the orders of winged insects (Insecta Pterygota, Fig 2) are chiefly based upon KRISTENSEN (1991 1994, 1995), with the following modifications (1) Plecoptera (stoneflies) are placed as sister group to the remaining Neopteran order (as suggested by KRISTENSEN 1991 1994, p 132, but not depicted in his cladogram, his Fig 5 5), (2) Hemiptera and Thysanoptera are treated as genuine sister groups (Superorder Condylognatha) following a more widely accepted hypothesis than joining the thysanopterans with psocodeans (Psocoptera + Phthiraptera), (3) Strepsiptera is placed as sister group of Coleoptera following KUKALOVA-PECK & LAWRENCE (1993) The alleged sister group relationship between Strepsiptera and Diptera (WHITING & WHEELER, 1994) has been seriously questioned by KRISTENSEN (1995)

Source MNHN, Pa s

The primary source for the phylogeny of the Hemiptera-Heteroptera (Fig 3) is the excellent monograph by SCHUH & SLATER (1995) supplemented by ANDERSEN (1982, 1995a), SCHUH (1986), SCHUH & STYS (1991), WHEELER *et al* (1993), and MAHNER (1993). The currently accepted classification of the Heteroptera excludes the southern temperate family Peloridae (Coleorrhyncha) from the suborder and divides it into seven infraorders (listed by the same order of families as in Fig 3) Enicocephalomorpha (Aenictopecheidae Enicocephalidae), Dipsocoromorpha (Ceratocombidae to Stemmocryptidae), Gerromorpha (Mesoveliidae to Gerridae), Nepomorpha (Nepidae to Helotrephidae), Leptopodomorpha (Aepophilidae to Leptopodidae) Cimocomorpha (Pachynomidae to Polyctenidae), and Pentatomomorpha (Aradidae to Rhopalidae) The relationships between these infraorders depicted in the cladogram (Fig 3) follows WHEELER *et al* (1993) and SCHUH & SLATER (1995).

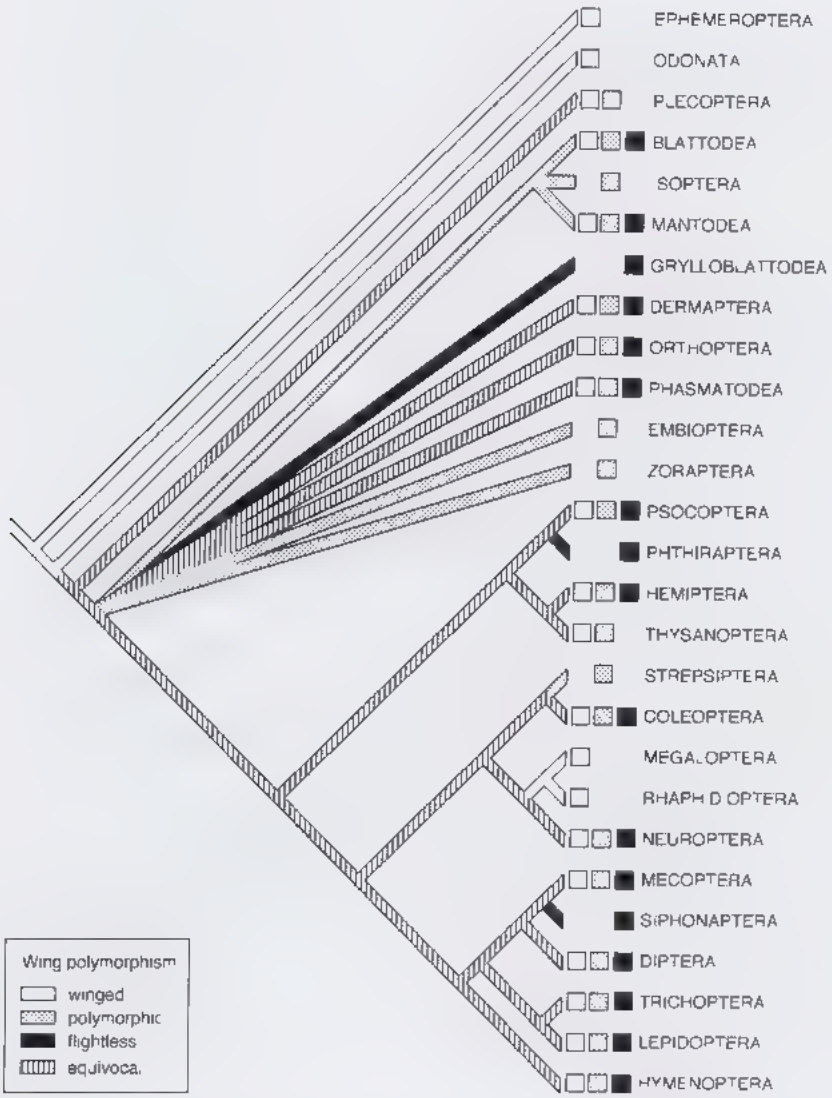

FIG 2 — Phylogeny and distribution of three states of wing development in the orders of pterygote insects Further discussion in text

Source MNHN Paris

The phylogenetic relationships between the genera of the family Gerridae (Fig 4) are primarily based upon MATSUDA (1960) and ANDERSEN (1982, 1995b, and unpublished) Finally, phylogenies for species or species groups of the genera *Aquarius*, *Gerris* and *Limnoporus* (Fig 5) are compiled from ANDERSEN (1990, 1993b) and ANDERSEN & SPENCE (1992) with modifications for the last mentioned genus as discussed by SPERLING *et al.* (in press)

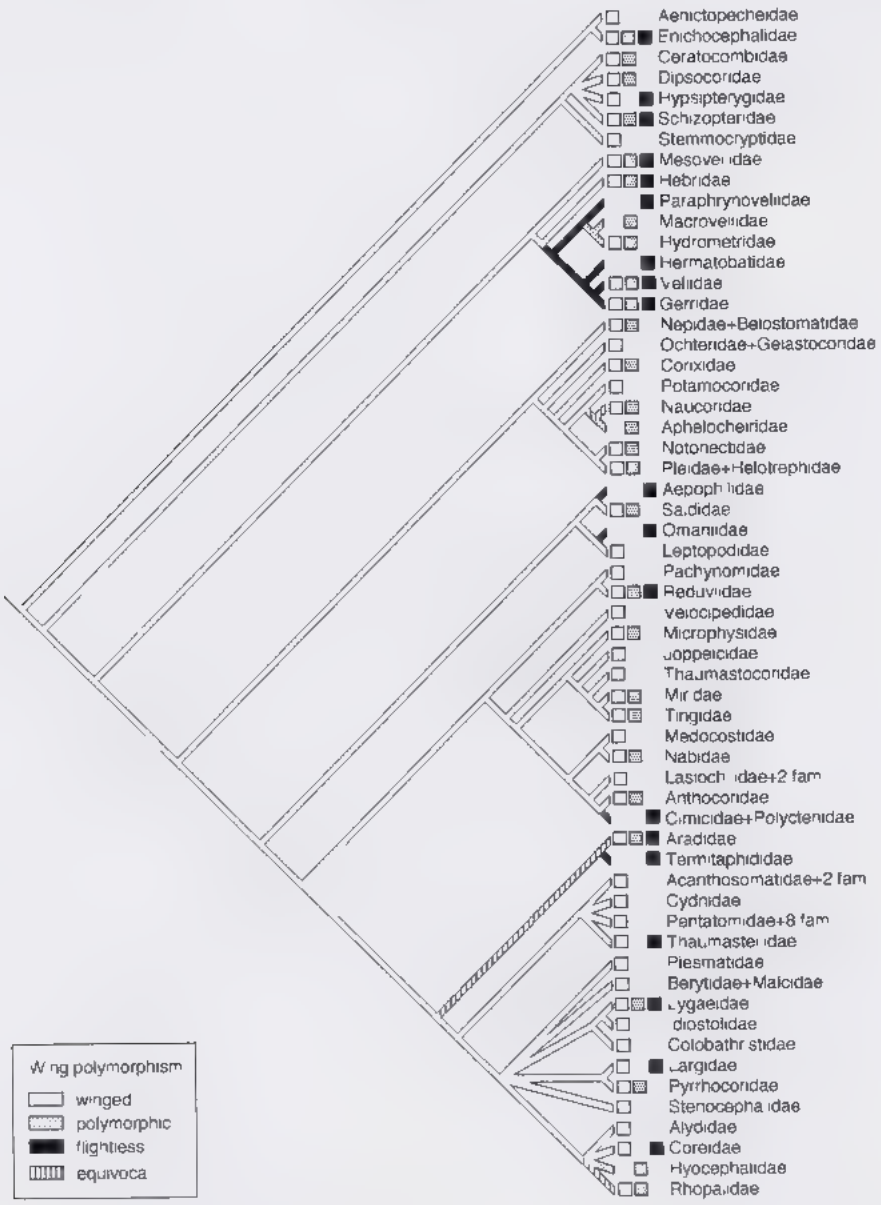

FIG 3 — Phylogeny and distribution of three states of wing development in the families of Hemiptera-Heteroptera Further discussion in text

Source MNHN, Paris

Wing morph frequencies

A complex terminology (*e g* , ANDERSEN 1982, SCHUH & SLATER, 1995) has been employed to distinguish various degrees of wing modification in flightless and polymorphic insects (apterous, brachypterous, etc) For the sake of simplicity, I here distinguish between three states of wing morphism in adult insect orders, Heteroptera, and the Gerridae (slightly different states are used for *Aquarius*, *Gerris*, and *Limnoporus*, see below) (1) *winged*, all individuals of a species possess fully developed and presumably functional wings, (2) *polymorphic*, natural populations composed of both winged and flightless adult individuals, the latter sometimes restricted to only one sex and (3) *flightless*, all individuals of a species flightless Information on the distribution and frequencies of different wing morphs are chiefly compiled from the following sources ROFF (1990) and CSIRO & NAUMANN (1991) for the orders of insects, SCHUH & SLATER (1995) for the Hemiptera Heteroptera; and ANDERSEN (1982, 1990, 1993a, 1993b, ANDERSEN & SPENCE, 1992) for the Gerridae

Optimization

The evolutionary changes between different states of wing development (treated as non additive or unordered) were optimized on the cladograms using the computer program MacClade, version 3 05 (MADDISON & MADDISON, 1992) with the "show all most parsimonious states at each node" option The results of this optimization are shown as different shading of the branches of the cladogram The shading for "equivocal" is used when the optimization is unable to resolve the state of wing development on a particular branch

RESULTS AND DISCUSSION

The orders of pterygote insects

In only 4 out of 27 orders of pterygote insects are the adult stage always winged These are the Ephemeroptera (mayflies) and Odonata (dragonflies, damselflies), previously united in the Palaeoptera, and the small endopterygote orders Megaloptera (alderflies, dobsonflies) and Rhaphidioptera (snakeflies, camelneckflies) Except for the last mentioned order, the immature stage is aquatic in these groups Flightless adult forms are rare in the Plecoptera (stoneflies), Neuroptera (lacewings), Mecoptera (scorpionflies), and Trichoptera (caddisflies) the first and last order with aquatic immature stages Relative to the total number of species, flightless or wing polymorphic species are also rare in the large orders Diptera, Lepidoptera, and Hymenoptera

Obligatorily flightless orders are the Phthiraptera (sucking and chewing lice, Anoplura and Mallophaga) and Siphonaptera (fleas), both ectoparasites on warm-blooded vertebrates, and the small northern temperate order Grylloblattodea.

In the remaining orders, flightlessness or wing polymorphism (sex-bound or not) is common or very common in the exopterygote orders Blattodea (cockroaches), Isoptera (termites, but limited to the worker caste), Mantodea (praying mantids), Orthoptera (grasshoppers, locusts, crickets), Dermaptera (earwigs), Phasmatodea (stick insects), Embioptera (web-spinners), Zoraptera, Hemiptera, and Thysanoptera (thrips) Relative to the total number of species, loss of the flight ability is uncommon in the Coleoptera, but because wing reduction rarely affects the elytra, the flightless adult form is difficult to recognize and its frequency may therefore be underestimated in beetles

Because of the extremely diverse nature of wing development in most insect orders, the results of the optimization of the three states on the phylogeny of pterygote insects are ambiguous (Fig 2) Because both palaeopteran orders are obligatorily winged, this undoubtedly was the ancestral state for the Pterygota The ancestral state for the neopteran orders is equivocal (winged or polymorphic) However, this ambiguity disappears if the Strepsiptera is treated as a subordinate group of Coleoptera (following CROWSON, 1955, and later writings), the winged state is then selected as the ancestral one The Dictyoptera *s l.* (Blattodea + Isoptera + Mantodea) were probably primitively wing polymorphic and the Paraneoptera (Psocodea + Hemiptera + Thysanoptera) likewise It is very unlikely that the ancestors of these groups were

Source MNHN Paris

obligatorily flightless since this requires that wings have evolved as (autapomorphic) character reversals in some lineages Finally, although the ancestral state for the Endopterygota is equivocal, it is predicted that analyses with more finely resolved phylogenies will show, that ancestral endopterygotes were winged like the ancestors of all endopterygote orders except the Strepsiptera and Siphonaptera

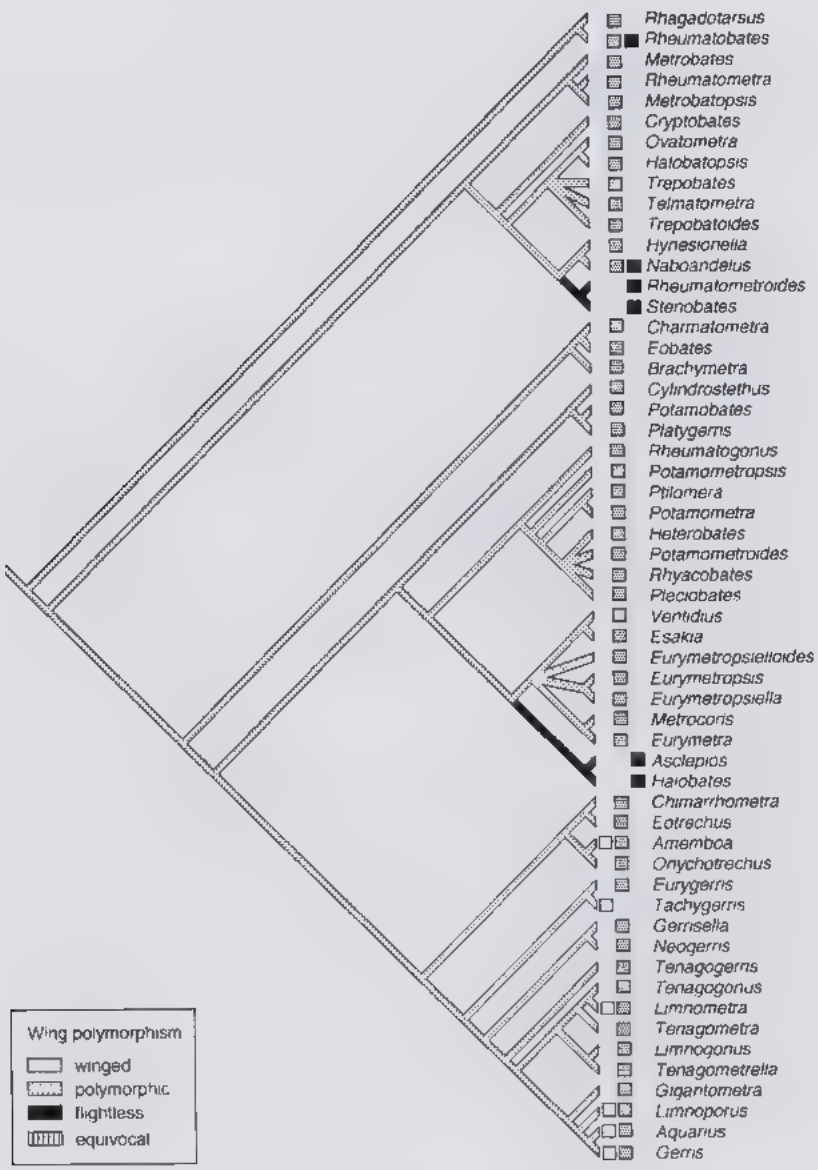

FIG 4 Phylogeny and distribution of three states of wing development in the genera of Gerridae (Hemiptera-Heteroptera) Further discussion in text

Source MNHN Paris

At the level of insect orders, phylogenetic inference leading to estimates of the causes underlying patterns of flightlessness is difficult However, orders with a gradual metamorphosis ("paurometabolous" orders) and with immatures and adults occupying similar niches are more frequently wing polymorphic or flightless than orders with complete metamorphosis and a significant niche shift between the immature and adult stage (ROFF, 1990) To the last category belongs the hemimetabolous orders Ephemeroptera, Odonata, and Plecoptera, and most of the holometabolous orders Wing polymorphism and flightlessness are also more frequent in orders where adult flight is not an "everyday" activity necessary for feeding, mating, or local dispersal (as the Orthoptera, Hemiptera, and Coleoptera).

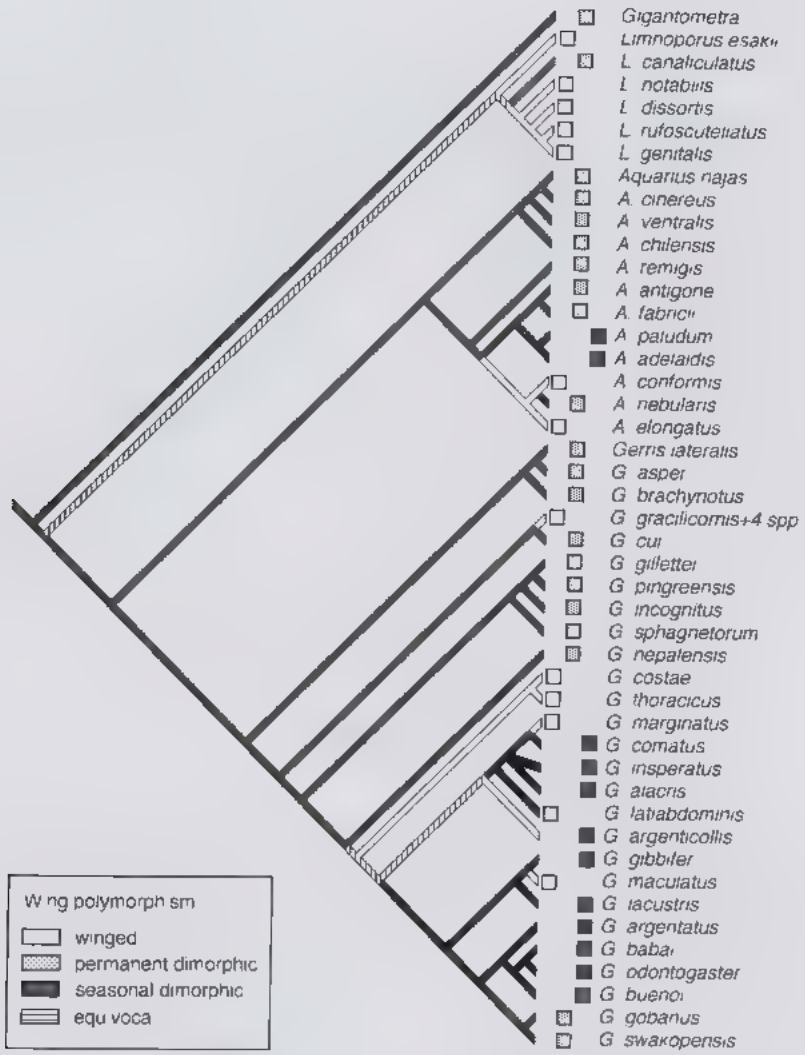

FIG 5 – Phylogeny and distribution of states of wing development in species belonging to *Aquarius*, *Gerris*, *Gigantometra*, and *Limnoporus* (Hemiptera-Heteroptera, Gerridae) Further discussion in text

Source MNHN Paris

The families of Hemiptera-Heteroptera

The Hemiptera (true bugs, cicadas, plant- and treehoppers, plantlice, etc) with about 82,000 described species is the largest of the hemimetabolous insect orders Wing polymorphism is very common and widespread in this order, in particular within the suborder Heteroptera or true bugs where it occurs in 41 out of 75 families (Fig 3) Flightlessness is especially common in the infraorders Enicocephalomorpha, Dipsocoromorpha, Gerromorpha (semiaquatic bugs), and in the large families Reduviidae (assassin bugs) and Miridae (plant bugs) belonging to the Cimicomorpha, whereas it is relatively rare in the Nepomorpha (aquatic bugs), Leptopodomorpha (shore bugs), and Pentatomomorpha except for the families Aradidae (bark bugs) and Lygaeidae (seed bugs) Obligatorily flightless species are found in the Paraphrynoveliidae, Cimicidae (bed bugs), and Polyctenidae (bat bugs), the last two families being vertebrate ectoparasites, the Termitaphididae (termite inquilines), and in the small marine families Hermatobatidae, Aepophilidae, and Omaniidae

An optimization of the three states of wing development on the reconstructed phylogeny of the Heteroptera (Fig 3) unequivocally picks the winged state as the ancestral one for all major lineages Thus, wing polymorphism and flightlessness have seemingly evolved independently numerous times in true bugs The flightless state is inferred to be ancestral to the families of Gerromorpha (except Mesoveliidae and Hebridae) The explanation for this result is the presence of two small, obligatorily flightless families (Paraphrynoveliidae and Hermatobatidae) as well as flightless taxa in the large families Veliidae and Gerridae

At the level of heteropteran families, phylogenetic inference leading to estimates of causality between flightlessness and other attributes is equally problematical Flightless forms are most frequent in predaceous bugs and in species inhabiting ground litter (Enicocephalidae, most Dipsocoromorpha, the gerromorphan families Mesoveliidae, Hebridae, Paraphrynoveliidae, Macroveliidae, some Leptopodomorpha and Cimicomorpha, and the Lygaeidae), semiaquatic habitats (most Gerromorpha), marine habitats (Hermatobatidae, some Veliidae and Gerridae, Aepophilidae, and Omaniidae), and in ectoparasites (Cimicidae, Polyctenidae, some Lygaeidae), and in those bugs which live under bark (Aradidae and Lyctocoridae) Winged forms are predominant in phytophagous bugs belonging to the family Miridae (Cimicomorpha) and most families of the Pentatomomorpha

The genera of Gerridae

The Gerridae (water striders) is one of the largest families of semiaquatic bugs (Heteroptera, infraorder Gerromorpha), with about 600 described species The vast majority of species belonging to this group are wing polymorphic (Fig 4) Only the genera *Amemboa* (Eotrechinae), *Tachygerris*, *Limnometra*, *Limnoporus*, *Aquarius*, and *Gerris* (Gerrinae) include species which are monomorphic winged Obligatorily flightless species are restricted to the genera *Rheumatometra* (Rhagadotarsinae), *Naboandelus*, *Rheumatometroides*, *Stenobates* (Trepobatinae), *Asclepios*, and *Halobates* (Halobatinae), all living in marine habitats (from estuaries, mangroves, and intertidal coral reef flats to the open sea)

Ancestral gerrids were probably wing polymorphic (like most gerromorphan bugs, see Fig 3) and the loss of the flightless form in some species is most likely secondary Since all marine water striders have polymorphic freshwater relatives, the complete loss of the winged form associated with the extremely stable marine habitats is easily explained Adult water striders only

Source MNHN, Paris

use flight for dispersal among habitats and (in temperate regions) in connection with hibernation which takes place on land (ANDERSEN, 1982)

The species of Gerris *and related genera*

Northern temperate water striders belonging to the genera *Aquarius*, *Gerris*, and *Limnoporus* (formerly united in the genus *Gerris s.l.*) have been extensively used as model organisms in studies of the relations between wing polymorphism, dispersal strategies, and life history dynamics (ANDERSEN, 1973, VEPSALAINEN, 1978, SPENCE, 1989; SPENCE & ANDERSEN, 1994) The phylogeny of the three genera is relatively well understood (ANDERSEN, 1990, 1993b, ANDERSEN & SPENCE, 1992) The cladogram (Fig 5) shows the relationships between most species belonging to the genera *Limnoporus*, *Aquarius*, *Gerris* with the monotypic genus *Gigantometra* added as outgroup (ANDERSEN, 1995b)

Water striders belonging to this group are either monomorphic winged or wing dimorphic Following ANDERSEN (1993a), I distinguish between permanent dimorphism in which populations usually include both winged adults (which may be rare) and adults with more or less reduced wings and flight musculature, and seasonal dimorphism in which the flightless adult form only occurs during summer, indicating a direct breeding second generation The wing development of the two types of flightless forms follow different ontogenetic pathways (Fig 1) In seasonally dimorphic species, the fourth (IV) and fifth nymphal instar (V) have the same size of wing-pads as nymphs from which the winged adults emerge (a $\rightarrow$ A' $\rightarrow$ A) In permanently dimorphic species, the fourth and fifth instar nymphs have distinctly reduced wing-pads (f $\rightarrow$ F $\rightarrow$ F)

Optimization of the three states of wing development on the cladogram (Fig 5) points to the permanently dimorphic state as the ancestral one for the whole group as well as for *Aquarius* and *Gerris*, while the wing morphism state is equivocal for the genus *Limnoporus* (one species dimorphic, the rest always winged)

Flightlessness and the "oogenesis-flight" syndrome

The concurrent development in insect ontogeny of the flight apparatus, the ovaries, and the ancillary systems such as the fat-body has been termed the "oogenesis-flight" syndrome (JOHNSON, 1969) The differential development of these systems in response to environmental factors has produced a variety of different forms in insects, ranging from sexually immature, migrant individuals, to various types of flightless, sexually mature, and eventually parthenogenetic or paedogenetic individuals The wing muscles are relatively massive structures, comprising 10-20 % of the body mass in most insects and undoubtedly consuming a significant proportion of an insect's energy budget (ROFF, 1990) Flightless female insects lack wing muscles and are therefore able to divert more energy to the production of oocytes In addition, winged females commonly autolyse (self-digest) their flight muscles during egg production as well documented in northern temperate water striders (ANDERSEN, 1973, 1982, FAIRBAIRN & DESRANLEAU, 1987; KAITALA & HULDÉN 1990)

In insects where adult flight is not an "everyday" activity necessary for feeding, mating, and escaping from temporary or deteriorating habitats, it can be predicted that patterns of flightlessness often will be compatible with the "oogenesis-flight" syndrome explanation The high incidence of flightless forms in the orders Orthoptera, Hemiptera, and Coleoptera, and the

Source MNHN, Paris

distribution of flightless forms among and within the families of Heteroptera seem to meet this prediction (SCHUH & SLATER, 1995)

Flightlessness and ectoparasitism

Ectoparasites on warm-blooded vertebrates belonging to the orders Phthiraptera, Siphonaptera, Hemiptera-Heteroptera, and Diptera, are all obligatorily flightless It has been suggested (LYAL, 1985) that the chewing lice (Mallophaga, a paraphyletic group) and sucking lice (Anoplura) evolved from some subgroup of the Psocoptera, namely the family Liposcelidae which contains many flightless species (booklice) If this hypothesis is correct, the phylogeny supports a scenario where loss of flight ability preceded ectoparasitism (Fig 6) However, the fact that some ectoparasitic Diptera (belonging to the family Hippoboscidae) are winged or dimorphic points at the opposite sequence of evolution, where the parasitic way of life preceded wing loss (WAGNER & LIEBHERR, 1992) In the case of Siphonaptera, its presumed sister group, the Mecoptera, contains both dimorphic and flightless forms Since ancestral scorpionflies most likely were winged, the loss of flight ability and ectoparasitism in fleas coincide and it is not possible to tell which came first The situation is the same for the heteropteran families Cimicidae

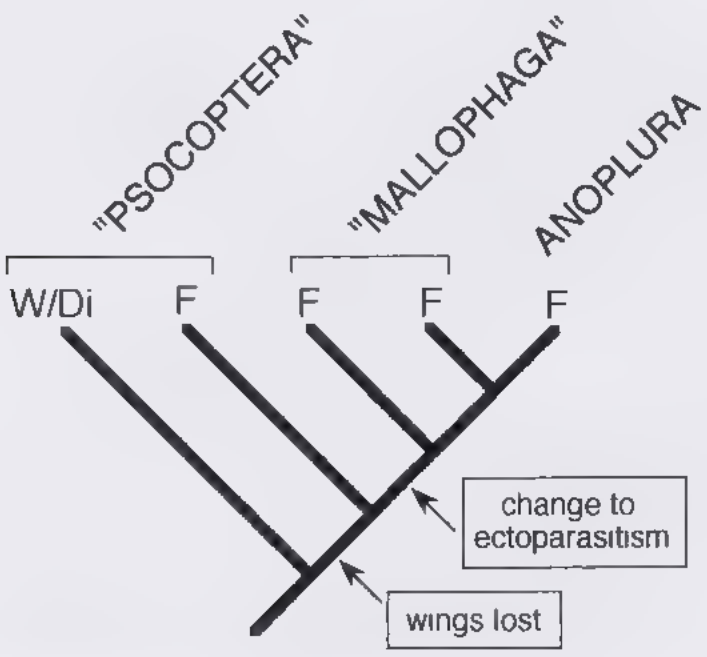

FIG 6 Presumed phylogeny and evolution of flightlessness and ectoparasitism in orders of Insecta-Psocodea Both Psocoptera and Mallophaga are paraphyletic in this phylogenetic hypothesis. Abbreviations Di, wing dimorphic, F flightless, W, winged. Further discussion in text

Source MNHN, Paris

and Polyctenidae which form a monophyletic group related to the Anthocoridae

Flightlessness and habitat stability

Phylogenetic tests of scenarios explaining the evolution of flightlessness in relation to habitat are difficult to perform at the level of insect orders as well as the families of Heteroptera ROFF (1990, 1994) found significant correlation between loss of flight ability with decrease in environmental heterogeneity, with increasing altitude and latitude, but not with isolation (*e.g.*, on oceanic islands) However, the correlation tests performed by ROFF (1990) did not take phylogeny into consideration. In a subsequent paper, ROFF (1994) tried to find methods for correcting his analyses for "phylogenetic effects" using the Orthoptera of North America as example However, the success of this trial was modest due to a lack of adequate phylogenies for this group of insects.

The currently accepted scenario for the evolution of flightlessness in insects (SOUTHWOOD, 1962, VEPSALAINEN, 1978; HARRISON, 1980, ROFF, 1986) assumes that winged monomorphism

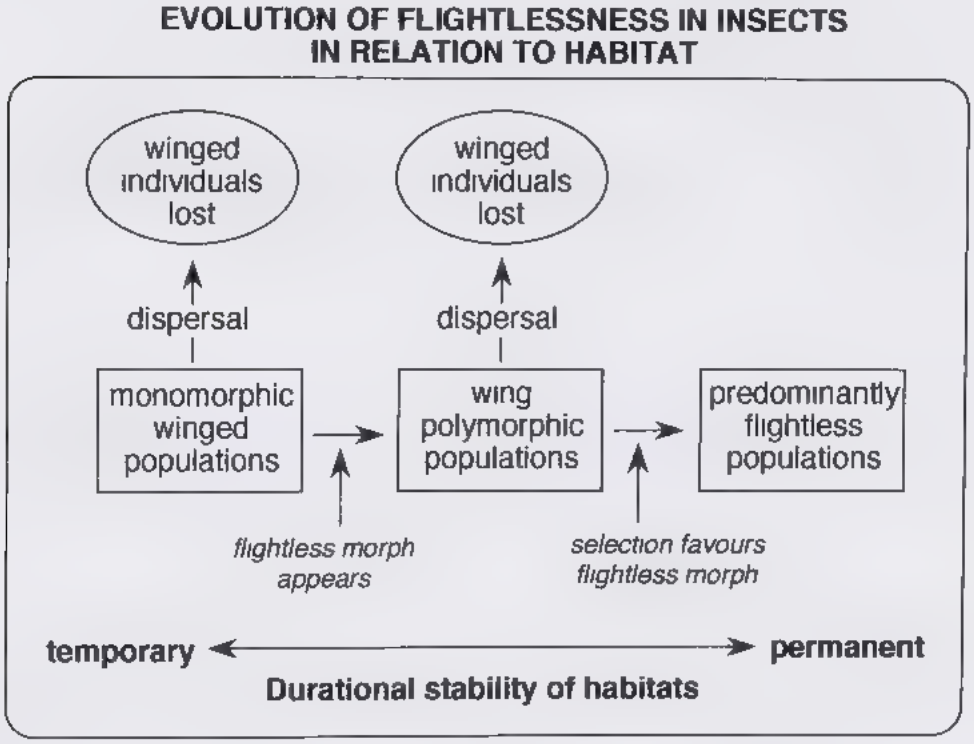

FIG 7 — Scenario for the evolution of wing polymorphism and flightlessness in relation to durational stability of habitats (from temporary to permanent). Evolutionary sequence from winged, through dimorphic, to predominantly flightless populations/species Further discussion in text

Source MNHN, Paris

is the original (ancestral) state, and that flightlessness and/or wing polymorphism has evolved through the combined effects of selection against dispersing winged individuals and presumed higher fitness value of non-dispersing, flightless individuals (Fig 7) In a changing environment where habitats proceed from being relatively unstable (temporary) to increasingly more stable (permanent), populations may change from being monomorphic winged to being wing dimorphic or even purely flightless If this scenario is applied to a monophyletic group of species which show various degrees of adaptation on a scale of environmental stability, it is predicted that winged species will occupy habitats placed towards the temporary end of the scale while wing dimorphic or flightless species will occupy habitats towards the permanent end of the scale A hypothetical phylogeny which meets this prediction is shown in Fig 8

Although this scenario has intuitive appeal, it should be submitted to phylogenetic tests using real insects before it is used as a general explanatory model for the evolution of flightlessness in relation to habitat Phylogenetic inference involving temperate water striders of the genera *Aquarius* and *Gerris* (ANDERSEN, 1993a), confirmed that patterns of wing polymorphism were related to habitat stability However, contrary to the predictions (Fig 8), the winged state is not the ancestral one Surprisingly, the ancestors are inferred to be predominantly flightless or permanently dimorphic (Fig 5), occupying relatively stable habitats Subsequent evolution of long-wingedness or seasonal dimorphism has allowed descendant taxa to colonize less stable habitats Thus, patterns of wing polymorphism in temperate water striders are more compatible with the hypothetical phylogeny shown in Fig 9

It is relatively straightforward to extend this approach to encompass other biological important traits beside the potential for dispersal, *e.g.*, fecundity, length of reproductive period, pressure from parasitoids and predators, reproductive strategies and mating systems (SPENCE, 1989, ANDERSEN, 1993a, 1994, 1996, SPENCE & ANDERSEN, 1994) Among other things, this approach allows predictions about where mechanisms of determination of adaptive traits may

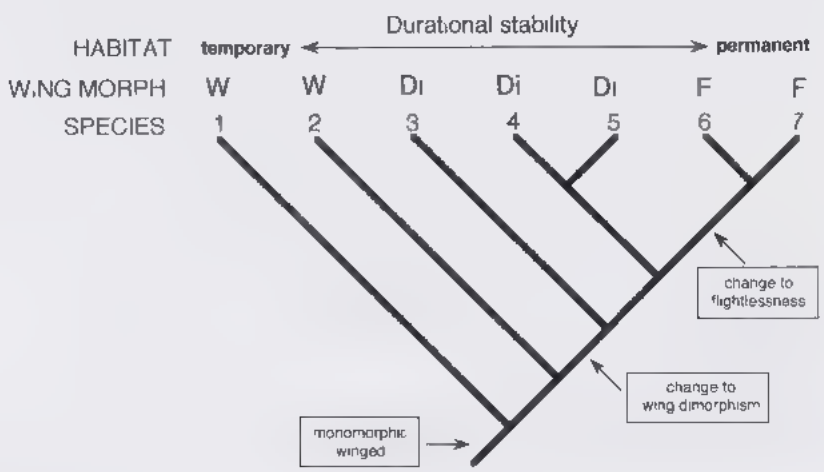

FIG 8 — Phylogenetical relationships between species 1-7 and patterns of wing morphism compatible with the scenario in Fig 7 Abbreviations Di wing dimorphic, F, flightless, W, winged adult morph Further discussion in text

Source MNHN, Paris

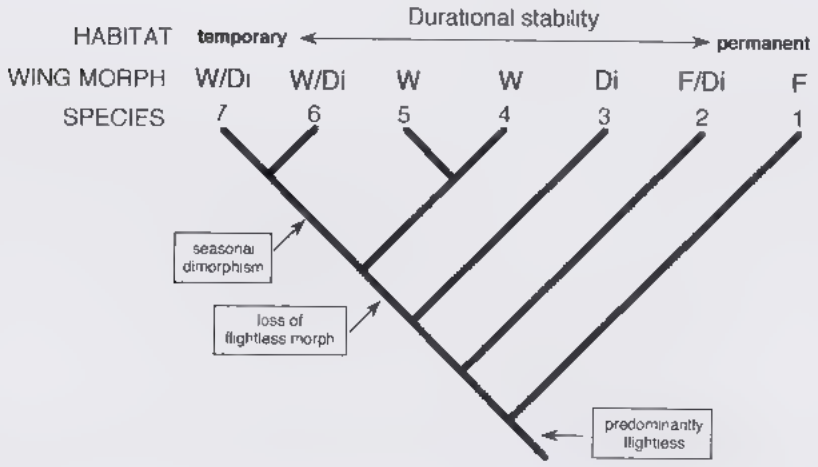

FIG 9 — Phylogenetical relationships between species 1-7 and patterns of wing morphism compatible with the observed pattern of wing polymorphism in northern temperate water striders belonging to the genera *Aquarius* and *Gerris* (see Fig. 5). Abbreviations as in Fig. 8. Further discussion in text.

differ and thus enable us to select the taxa appropriate for generalizations about selective processes leading to a particular kind of adaptation, like flightlessness and wing polymorphism in insects.

CONCLUSIONS

Wing polymorphism and flightlessness are very widespread among the orders of pterygote insects, in particular those orders where adult flight is not an "everyday" activity necessary for feeding, mating, or local dispersal, as in the Orthoptera, Hemiptera, and Coleoptera. Flightlessness is rare where there is a significant niche shift between the immature and adult individual, as in the Ephemeroptera, Odonata, and Plecoptera, and the holometabolous orders Diptera, Lepidoptera, and Hymenoptera. Because most orders of pterygote insects and many families of Hemiptera-Heteroptera are highly variable with respect to wing morphism, phylogenetic inference is not very helpful in disclosing the causes underlying the observed patterns of flightlessness. In general, however, the patterns are compatible with the "oogenesis-flight" syndrome explanation when viewed in a phylogenetic context.

At the generic level of the water strider family Gerridae, the ancestral mode of adaptation was wing dimorphism, and the few cases of winged monomorphism are estimated to be results of secondary loss of the flightless form. As an extension of permanent dimorphism in freshwater species, marine water striders are obligatorily flightless.

Ectoparasites on warm-blooded vertebrates (Phthiraptera, Siphonaptera, some Hemiptera-Heteroptera and Diptera) are all obligatorily flightless. If the chewing lice (Mallophaga) and sucking lice (Anoplura) evolved from some subgroup of the Psocoptera, the phylogeny supports

Source: MNHN, Paris

a scenario where loss of flight ability preceded ectoparasitism whereas the opposite sequence of evolution may apply to the Diptera-Hippoboscidae.

The currently accepted scenario for the evolution of flightlessness in insects assumes that the winged state is ancestral and that wing polymorphism or flightlessness have evolved through the combined effects of selection against dispersing winged individuals and presumed higher fitness value of non-dispersing, flightless individuals. The present study suggests that this scenario should be submitted to phylogenetic tests using real insects before it is used as a general explanatory model for the evolution of flightlessness in relation to habitat. For example, patterns of wing polymorphism in temperate water striders are more compatible with the reverse scenario, where ancestors are predominantly flightless or permanently dimorphic, occupying relatively stable habitats, and their descendants are long-winged or seasonally dimorphic, colonizing less stable habitats.

Finally, the present study emphasizes the need for taking phylogeny into consideration for further understanding of the evolution of flightlessness in insects. It also illustrates the importance of choosing the right taxonomic level and phylogenetic resolution for analysis. When correctly applied, phylogenetic inference applied to patterns of biologically important attributes can make significant contributions towards understanding the causes underlying these patterns and suggests possibilities for process. In the end, however, understanding process will depend on studying process directly.

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Source: MNHN, Paris

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Habitat and Ant-Attendance in Hemiptera : a Phylogenetic Test with Emphasis on Trophobiosis in Fulgoromorpha

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ABSTRACT

The biological attribute "ant-mutualism" is so widely distributed within the Hemiptera that SCHAEFER (1987) suggested that the association of Hemiptera with ants represents the retention of an early way of life which originated on the ground and always preceded a life up on plants A phylogenetic test of SCHAEFER's scenario indicates that ant-mutualism cannot be retained as ancestral for all major hemipteran clades but arose independently several times in groups for which habitat is above ground Causation between a ground habitat and ant-attendance is not corroborated Special attention is paid to trophobiosis in Fulgoromorpha Although all planthoppers could theoretically be associated with ants only a few of them are ant-attended, and mainly the Tettigometridae Members of this family share morphological characteristics (no jumping apparatus, no wax plates, no sensory pits, a long anal tube, no anal combs no anal apodemes in adults) and particular fulgoromorph behavior traits (subsociality sessile behavior) that could have evolved under selection for trophobiosis This calls for a reanalysis of these morphological and behavioral characters that have been generally considered as plesiomorphic Durable fulgoromorphan-ant associations are observed when planthoppers are unable to escape or live with gregarious or subsocial behaviors

RÉSUMÉ

Habitat et relations avec les fourmis chez les Hemiptères (plus particulierement les Fulgoromorphes) : un test phylogénétique

L'attribut « mutualisme avec les fourmis » a été si souvent observé chez les Hémiptères que SCHAEFER (1987) a suggéré que l'association Hemiptères-fourmis serait l'expression d'un mode de vie ancestral ayant pris place au niveau du sol avant que ces insectes ne conquièrent les strates superieures de la vegetation Un test phylogenetique refute le scenario de SCHAEFER et montre que le mutualisme avec les fourmis ne peut etre retenu comme un etat ancestral pour tous les grands clades d'Hemiptères Au contraire il serait apparu de maniere independante à plusieurs reprises chez des groupes occupant déjà une strate de vegetation superieure La relation de causalité entre vie au niveau du sol et mutualisme avec les fourmis n'est donc pas retenue La trophobiose chez les Fulgoromorpha est plus particulierement abordee Bien que théoriquement tous les Fulgoromorphes puissent maintenir des relations trophobiotiques avec les fourmis seules quelques especes sont concernées et tout particulierement les Tettigometridae Cette famille presente des caracteristiques morphologiques (pas d'appareil de saut, pas de plaques cirieres ni de fossettes sensorielles, un tube anal allongé absences des processus pectinés et des apodemes anaux chez l'adulte) et ethologiques (comportements subsocial et faible mobilité) qui auraient pu être selectionnées dans le contexte du comportement de trophobiose Ceci plaide pour une nouvelle etude de ces caracteres morphologiques et appelle à vérifier leur homologie primaire supposée avec les etats plésiomorphes observés chez les Cicadomorphes Seuls les

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Source MNHN, Paris

Fulgoromorphes qui sont subsociaux et ou ne peuvent s'éloigner des fourmis semblent présenter des associations durables avec les fourmis

INTRODUCTION

One of the major problems in evolutionary biology is the impossibility of repeating speciation experiment Evolution is a historical process with a unique result, and the repetition of an experiment which validates the scientific results is inapplicable in this field of study Evolutionary biologists thus have substituted for the repetition of experiment the repetition of observation How those observations are organized and studied are the object of comparative biology, the purpose of which is a better understanding of "the extent and pattern of organic diversity" (HUEY, 1987)

Until the last two decades, diversity of a biological trait was most often analyzed according to a "horizontal analysis" the different states of the trait were observed and a scenario was inferred according to an *a priori* general idea of evolution of the group In such a view the proposed scenario took into account at the same time the observed states of the trait (pattern), the mechanisms which select the trait and those involved for its maintenance (processes) and it was impossible to distinguish between them Patterns and processes were merged in the same explanation Since then, one has seen increasing concern of taking into account the historical dimension in comparative biology Analysis of the diversity of a biological trait has now to be rooted in the phylogeny according to a "vertical analysis" The result of this has been the shift from empirical methods to analyze a biological trait to more formalized ones The former were producing a "series of natural histories" incorporating *ad hoc* explanations which were difficult to evaluate The latter now result in refutable "evolutionary scenarios" directly linked with phylogenetic patterns (FUNKS & BROOKS, 1990, BROOKS & MCLENNAN, 1991,)

To study and compare biological traits (morphological, physiological, behavioral or ecological) between different taxa, two main types of methodologies have been developed The first uses statistical techniques (RIDLEY, 1989, FELSENSTEIN, 1985, HARVEY & PAGEL, 1991), the second consists of mapping the traits being studied onto cladograms (CODDINGTON, 1988, BROOKS & MCLENNAN, 1991, GRANDCOLAS *et al.*, 1994, ANDERSEN, 1995,) In this last case, patterns of biological traits are produced and used to test proposed models of evolutionary processes If the model fits the pattern observed then it is corroborated Indeed, such procedures do not aim to explain how evolution has proceeded (processes) but seek to describe or to account for what has happened (patterns) Patterns and processes provide each by themselves a better understanding of evolution but any explanation by processes using models needs to fit with what has happened as it is shown by patterns In such a way, phylogenetic patterns test evolutionary scenarios proposed by models or allow one to infer new evolutionary scenarios waiting for models (ELDREDGE & CRACRAFT, 1980, CARPENTER, 1989, GRANDCOLAS *et al.*, 1994) All these methodologies provide new insights into the origin and the development of biological traits and more generally they are concerned with the origin and the development of biodiversity Revisiting old well-established ideas using these new approaches has most often raised new and sometimes unexpected interpretations about different aspects of evolution *e g.* cave adaptation and return to epigean life, (DESUTTER-GRANDCOLAS, 1994) or social behavior and return a solitary way of life (PACKER, 1991)

Source MNHN Paris

With special attention paid to the Auchenorrhyncha and the Fulgoromorpha within the Hemiptera, the first aim of this paper is to revisit the interpretation of causation (GRANDCOLAS *et al.*, 1994) given for two biological attributes (*sensu* MICKEVICH and WELLER, 1990) "habitat" and "ant-attendance". Ant-attendance, or trophobiosis, is so widely distributed within the Hemiptera that SCHAEFER (1987) has suggested that the association of Auchenorrhyncha with ants represents the retention of an early way of life and is "a secondary consequence" of the ancestral habitat (ground level) of the Auchenorrhyncha (SCHAEFER, 1981, 1987). The second aim of this work is to provide the basis for new research directions on ant-fulgoromorph mutualism with special attention paid to the Tettigometridae on both morphological and behavioral particularities of this family.

MATERIALS AND METHODS

Methodology

Both "habitat" and "trophobiosis" are two traits for which homology is difficult to establish. They cannot be directly used in constructing phylogenies where an hypothesis of primary homology has to be proposed first for the putative synapomorphies (DE PINNA, 1991). They are used here as "attributes", according to the meaning of MICKEVICH and WELLER (1990). The mapping methodology has been used in this work. All trait states are unordered and Wagner parsimony (FARRIS 1970) is used to optimize them onto the cladogram using MacClade, version 3.06, (MADDISON and MADDISON, 1992).

To test if there is any relation between the habitat and its changes and evolution of trophobiosis in the focal group, the following protocol was employed:

1 Mapping the different habitats observed onto a phylogeny (DELEPORTE, 1993) and inferring historical changes to determine the ancestral state.

2 Mapping trophobiosis onto the same phylogeny and inferring the ancestral state of this attribute.

3 Inferring from the changes observed at the different nodes how the two attributes "ant-attendance" and "habitat" are linked onto the cladogram and whether there is any indication of causation or relationship (GRANDCOLAS *et al.*, 1994) between the two traits.

Phylogenetic background

Hemipteran and Fulgoromorphan phylogenies have been widely recast in the last few years and differ substantially from the classic view summarized by EVANS (1963, 1977) and used by SCHAEFER (1987). This results from recent phylogenetic works dealing with morphological (ASHLOCK, 1988, EMELJANOV, 1987, 1990, DIETRICH & DIETZ, 1993, BOURGOIN, 1993b) and/or molecular data (WHEELER *et al.*, 1993, SORENSEN *et al.*, 1995, CAMPBELL *et al.*, 1994, 1995, VON DOHLEN and MORAN, 1995, BOURGOIN *et al.*, 1997). Homoptera is no longer considered as a monophyletic group (SORENSEN *et al.*, 1995) and even the monophyly of the Auchenorrhyncha is now questionable (BOURGOIN, 1993b, CAMPBELL *et al.*, 1995). Within Fulgoromorpha, the basal position of the Tettigometridae has been recently debated: morphological and molecular evidence now places this clade among more recent lineages (BOURGOIN *et al.*, 1997). The classical (e.g. SCHAEFER, 1987) and the revised (represented by the consensus of these recent cladistic analyses) Hemiptera and Fulgoromorpha phylogenies have been both tested in this study. According to SORENSEN *et al.* (1995), Euhemiptera refers to the monophyletic group [Cicadomorpha + Neohemiptera] and Neohemiptera to [Fulgoromorpha + Coleorhyncha + Heteroptera].

Attributes: habitat and ant-attendance

The different states of the attributes "habitat" and "ant-attendance" for the major taxa of Hemiptera and for the Cicadomorpha and Fulgoromorpha families are provided in Table I. Nymphal habits, when known, have been chosen first to determine the state of the habitat attribute in the different lineages. This assumes that nymphs retain more specific information ("more conservative", SCHAEFER, 1987), than adults which are more likely to expand their habitat and their range of host plants as also noted by WILSON *et al.* (1994). Most information comes from SCHAEFER (1987) with some modifications. Data have been completed mainly for the Fulgoromorpha using WILSON *et al.*'s (1994) important paper for the habitat. For Tettigometridae, it has been reported several times that they have been found underground attended by ants. This has led to the idea that tettigometrid nymphs typically live on plant roots (EMELJANOV, 1987), a view that has been widely accepted (O'BRIEN & WILSON, 1985, WILSON *et al.* 1994) although, in fact, most nymph and adult tettigometrids live on and more generally above ground (most Hildinae and Egropinae, many Tettigometrinae, BOURGOIN, unpublished data). Therefore tettigometrid habitat has been coded as polymorphic. Even if some cercopoid nymphs are well known to occur in masses of froth on low grasses or in fluid-filled tubes (Machaerotidae) such behavior is most probably derived (BOLLARD, 1991). According to MAA (1963) and SCHAEFER (1987), Cercopoidea are considered as originally subterranean (many cercopid and

Source: MNHN, Paris

aphrophorid nymphs) as Cicadoidea nymphs. Heteroptera and Coleorhyncha are considered to live on the ground (SCHAEFER, 1981; SCHUH & SLATER, 1995), whereas habitat of Sternorrhyncha as above ground (FOLDI, REMAUDIERE, pers. com., and FOLDI, 1997 in this volume).

Trophobiosis in Auchenorrhyncha is documented by DIETRICH & MCKAMEY (1990), BUCKLEY *et al.* (1990), ROZARIO *et al.* (1992, 1993). In Cicadellidae, ant-leafhopper mutualism is rare and scattered among several subfamilies

TABLE 1 — General habitat and ant-attendance in major Auchenorrhyncha taxa. Data sources in text.

Taxa	Habitat	Ant-attendance
Sternorrhyncha	above ground	not in basal groups
Cicadomorpha		
Aetalionidae	above ground	+
Cercopoidea	in or on ground	-
Cicadoidea	in ground	-
Cicadellidae (other)	above ground	-
Eurymelinae	in, on or above ground	some species
Macropsinae	above ground	some species
Melizoderidae	?	?
Membracidae	above ground	+
Fulgoromorpha		
Achilidae + Achilixiidae	above ground, under bark	-
Cixiidae	in ground	some species, in ant nests
Delphacidae Ugyopini	on ground (plant crown)	-
Delphacidae Asiracini	on ground (plant crown)	-
Delphacidae (other)	on ground (plant crown)	some species, under ant shelters
Derbidae	above ground, under bark	-
Dictyopharidae	above ground	-
Eurybrachidae	above ground	-
Flatidae	above ground	-
Fulgoridae	above ground	-
Gengidae	?	?
Hypochthonellidae	in ground	in ant nests
Issidae + Acanaloniidae	above ground	some species
Kinnaridae + Meenoplidae	in ground (kinnarids)	-
Lophopidae	above ground	-
Nogodinidae	above ground	-
Ricaniidae	above ground	-
Tettigometridae	in, on or most often above ground	most often outside ant nests
Tropiduchidae	above ground	-
Coleorhyncha	on ground	-
Heteroptera	on ground	not in basal groups

Source: MNHN, Paris

DIETRICH & MCKAMEY (1990), reporting the cases already known have shown that although most of the species in which ant-mutualism occurs belong to subfamilies generally thought to be old lineages (NIELSON, 1985), their apparent taxonomic disjunctions among Cicadellidae suggests multiple origins of these behaviors Indeed, several cases of ant-mutualism are reported in eurymeline and macropsine leafhoppers which are considered to have a relative basal place in the Cicadellidae phylogeny This information has been incorporated into the study by including a polymorphic status for these two taxa in regard to ant-attendance The other Cicadellidae subfamilies (Agallinae, Iassinae, Hecalinae, Idiocerinae), in which only one or two cases of ant-attendance have been reported, are not included in this study Excepted for Tettigometridae, trophobiosis in Fulgoromorpha is poorly documented (see further the second part of this study) and also scattered among different families Treating ant-attendance as a polymorphic attribute has been used for the Fulgoromorpha Cixiidae, Delphacidae and Issidae In Delphacidae where the phylogeny is best known the first divisions Ugyopini and Asiracini (*sensu* ASCHE, 1990, but see Emeljanov, 1995) have been used Trophobiosis in Sternorrhyncha and Heteroptera is restricted to non basal taxa (Sternorrhyncha and Heteroptera phylogenies according to CAMPBELL *et al* (1994) and WHEELER *et al* (1993) respectively) and thus without incidence possible on the ancestral state of this attribute which then has been considered as absent for these groups in this study

RESULTS AND DISCUSSION

Habitat

Mapping the attribute habitat onto the revised phylogeny (Fig 1) leads to an equivocal result for the Hemiptera and the three states in, on or above ground are equally parsimonious (7 steps each) Moreover, one cannot propose any ancestral state for the Euhemiptera, the Cicadomorpha, the Neohemiptera or the Fulgoromorpha In order to try to resolve these equivocal results and because the sister group of the Hemiptera is still unclear (KRISTENSEN, 1995), a basal group to the Hemiptera has been added permitting a test of the three different attribute states (Fig 2, test 1, 2 ,3) All trees are equal with a five step length An "above ground" basal state does not resolve the equivocal results (Fig 2a) An "on ground" basal state provides a full resolution the ancestral state for the Euhemiptera, the Neohemiptera and the Fulgoromorpha is "on ground", while it is in ground for the Cicadomorpha (Fig 2b) With an "in ground" basal state, the Neohemiptera and the Fulgoromorpha are left with an equivocal result between on or in ground, while the ancestral state for Cicadomorpha remains "in ground" (Fig 2c)

The putative sister groups for the Hemiptera are either the [Psocodea + Thysanoptera] or the Thysanoptera alone (KRISTENSEN, 1995) Insects in these two lineages live generally on ground (Psocodea, Terebrantia) but some live also above ground This last state does not allow to resolve the equivocal results If one retains an ancestral state on the ground for the Hemiptera, there is no equivocal result and one can suggest the following evolutionary scenario for the changes of habitat (Fig 2b) The ancestral habitat state for the Hemiptera was probably on ground and each major lineage has evolved in its own direction, whether above ground in Sternorrhyncha, in ground in Cicadomorpha (nymphs) or on ground in Neohemiptera In Cicadomorpha, the Membracoidea (*sensu* DIETRICH & DEITZ, 1993) have evolved to an above ground habitat for all instars In Fulgoromorpha one lineage (Delphacidae, Cixiidae, Kinnaridae-Meenoplidae) has moved to an underground habitat (Cixiidae and Kinnaridae-Meenoplidae nymphs) and a second lineage has changed to a complete above ground life This evolutionary scenario agrees with EMELJANOV (1987) rather than WILSON *et al.* (1994) who retain an ancestral subterranean feeding for the Fulgoromorpha and Auchenorrhyncha as a whole

Source MNHN, Paris

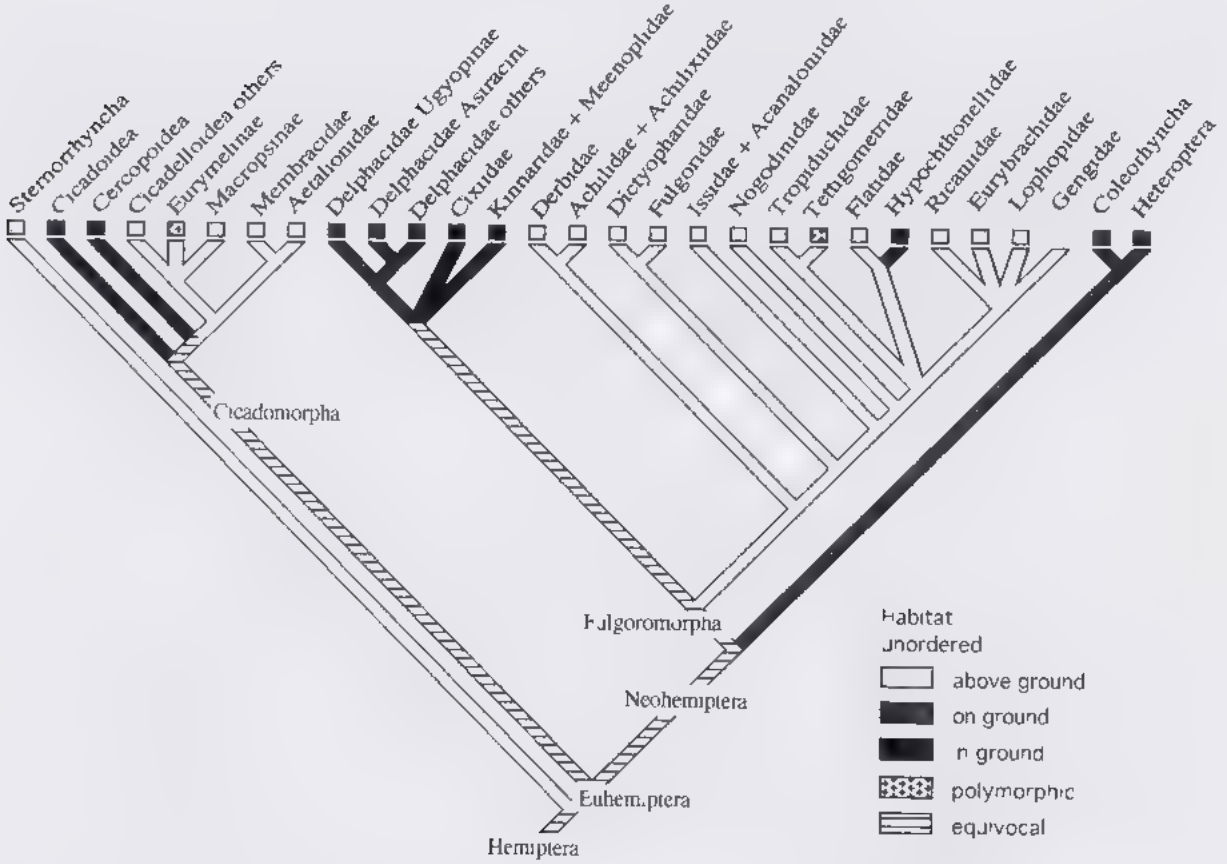

FIG 1 — Optimization of the habitat attribute (in, on or above ground) upon the phylogeny of the Hemiptera

Ant-attendance

The ancestral state of this attribute corresponds to an absence of ant-attendance for all the major lineages Hemiptera, Cicadomorpha, Euhemiptera, Neohemiptera and Fulgoromorpha (Fig 3) Within these clades each subgroup exhibiting trophobiosis has acquired this behavior independently except for the clade Aetalionidae + Membracidae This last result needs however to be confirmed Several taxa of membracids are known to be unattended by ants and an ancestral trophobiosis condition in Membracidae may not be retained if these taxa are confirmed as basal taxa Indeed, recent results of DIETRICH & DEITZ's (1993) phylogeny combined with WOOD's observations (1984) show for instance that Stegaspidini in the basal Stegaspidinae are unattended Ant-attendance in Fulgoromorpha is scattered throughout the taxa

SCHAEFER's scenario

SCHAEFER (1981) suggested that the original habitat of Hemiptera was on the ground He considered that, from a ground-dwelling hemipteran ancestors, two basic stocks emerged One became predacious and developed into the Heteroptera, the other lineage became phytophagous,

Source MNHN Paris

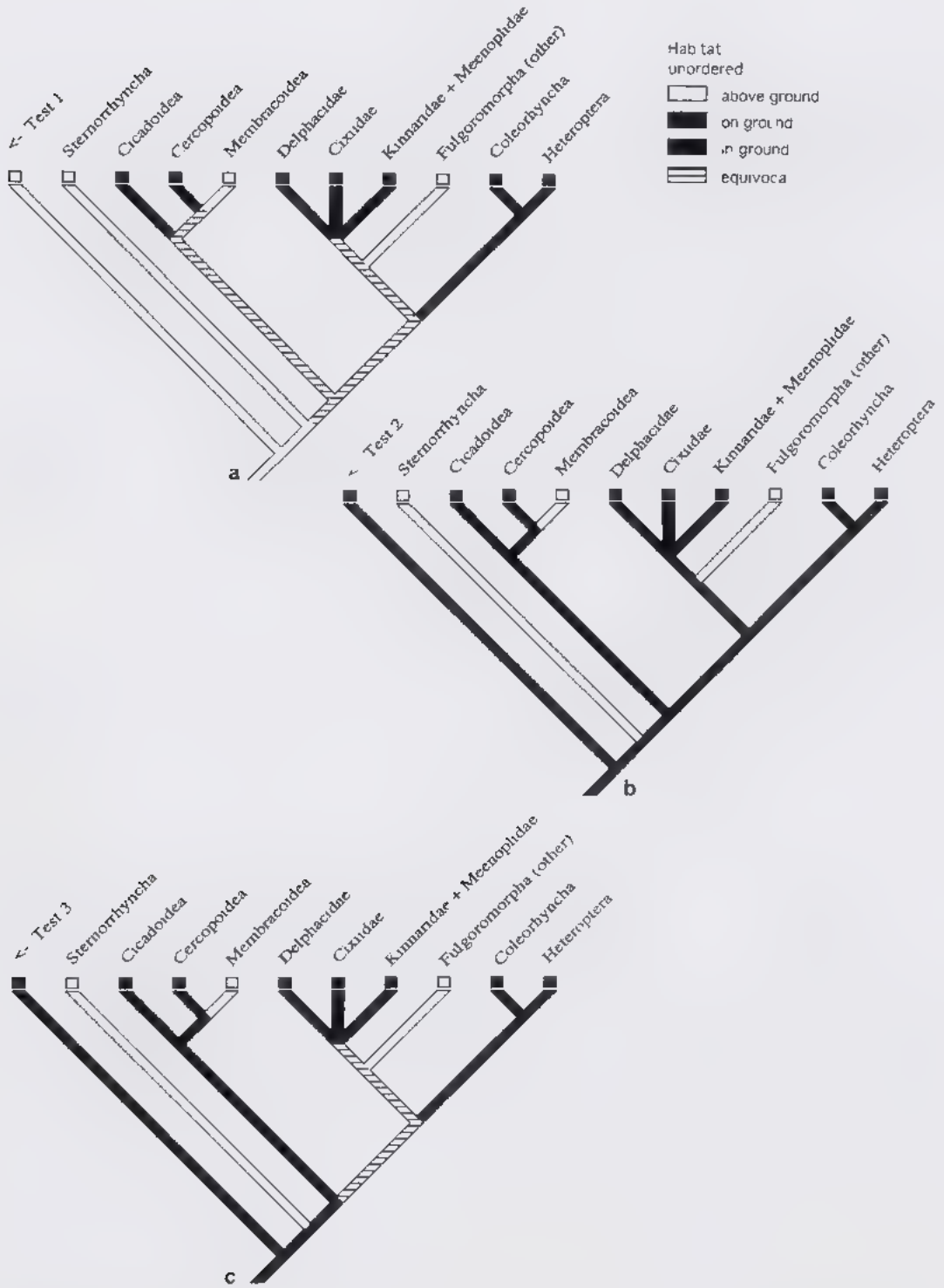

FIG 2 — Tests for optimization of the habitat attribute upon the phylogeny of Hemiptera inferring each habitat state possible at the base of the tree. Each cladogram is equally parsimonious to the others but only one (Test 2) allows a non equivocal resolution for this attribute. **a**: Test 1 above ground, **b**: Test 2 on ground, **c**: Test 3 in ground.

Source: MNHN, Paris

sucking plant juices (probably from roots), and developed into the Homoptera (SCHAEFER,1987) As many members of the Hemiptera, " especially many evolutionarily older members, live in ground debris, or on just below the surface of the ground, or associated with ground biota (such as roots, ants, burrowing mammals), or in groundlike habitats (*e g.* in nests, under bark)", this type of habitat "represents the retention of an early way of life, [ ] it is probable that the association with ants originated on the ground, and always preceded a life up on plants" (SCHAEFER, 1987).

SCHAEFER's scenario is thus built on three successive steps 1 Hemiptera lived ancestrally on the ground, 2 Ant attendance was an ancestral attribute for Auchenorrhyncha, and 3 Ant attendance preceded change of habitat from in/on ground to above ground However the last two steps are refuted and ant-attendance has appeared several times independently after all clades have moved above ground Indeed and according to the phylogeny used by SCHAEFER (Fig 4), mapping and optimizing parsimoniously the different types of habitat observed shows that it is impossible to decide if the state in, on or above ground (9 steps each) represents the ancestral state in the Hemiptera For the second attribute the ancestral state corresponds to an absence of ant-attendance for all the major lineages Hemiptera, Auchenorrhyncha, Cicadomorpha, Neohemiptera, Fulgoromorpha

Why do we obtain different results? To address this scenario SCHAEFER has clearly tried to link his argument with the historical perspective according to three main points The first one is in accordance with the methodology used here the phylogenetic pattern allows one to choose between plesio-apomorphic states of the character under study using a parsimonious optimization However SCHAEFER has added two more points to build his scenario "ingroup commonality criterion" and "older characters are primitive" Unfortunately, these criteria are well known to be inappropriate and should not be used to address character polarity (HENNIG, 1966, NELSON & PLATNICK, 1981, WATROUS & WHEELER, 1981, and reviews in BROOKS & MACLENNAN, 1991 or FOREY *et al.*, 1992) Using these criteria and adding *ad hoc* hypotheses lead to build a scenario out from parsimony and to rend it unrefutable

Ant-attendance and habitat in the Hemiptera

In conclusion, which scenarios can be proposed for these two attributes according to the above analyses? A parsimonious account of the patterns observed for these two attributes is proposed here with some *hypotheses* on the processes which could have been involved to explain these patterns These are not *ad hoc* arguments but just possible explanations that still need to be tested

With SCHAEFER (1987) one can retain a ground level habitat as an ancestral condition in the Hemiptera as a whole One may expect that for competitive reasons (?) each major lineage has evolved by itself either on ground (Neohemiptera), above ground (Sternorrhyncha) or in ground (Cicadomorpha) Probably with the evolution of lignophytes leading to angiosperms that allowed new feeding strategies passing from non-phloem to phloem feeding (CAMPBELL *et al.*, 1994, 1995), each group had the opportunity to radiate independently in an above ground level

Source MNHN, Paris

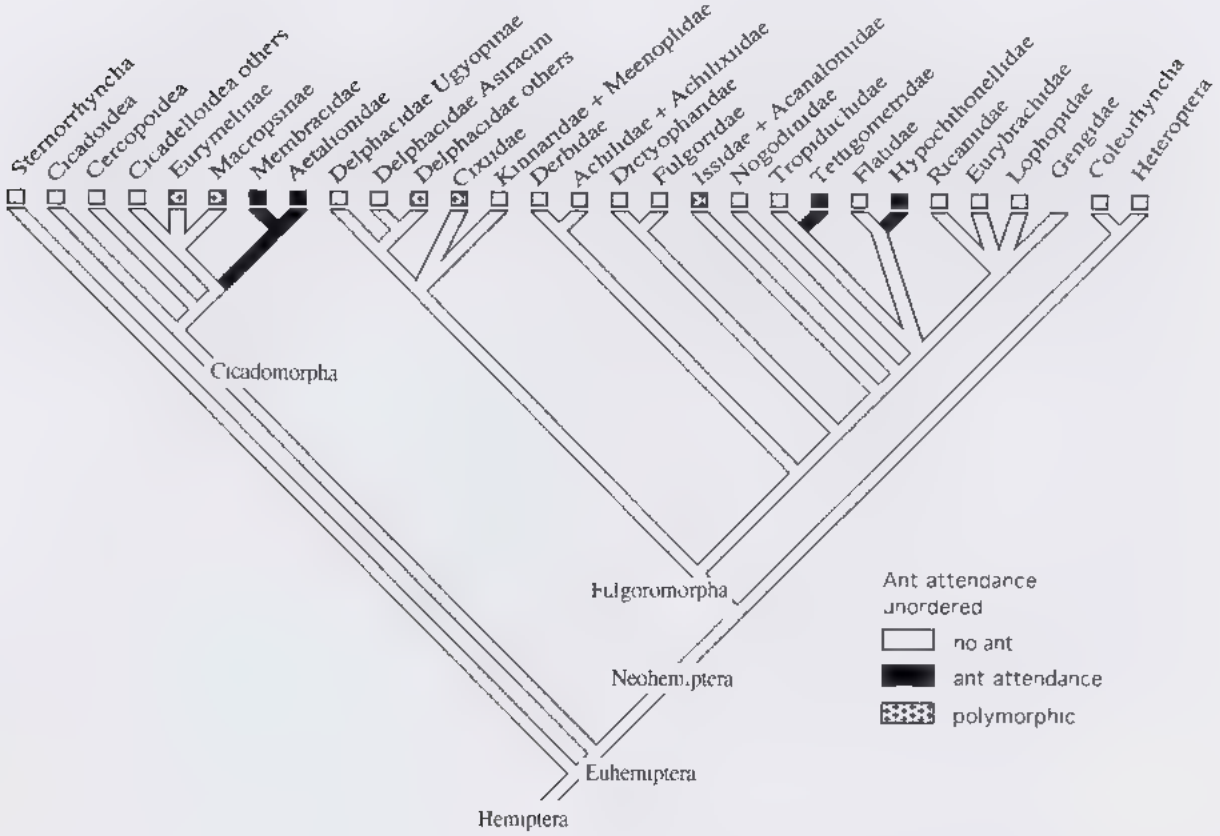

FIG. 3 — Optimization of the ant-attendance attribute upon the phylogeny of the Hemiptera

recent Sternorrhyncha, Membracoidea, higher Fulgoromorpha and secondary phytophagous Cimicomorpha and Pentatomorpha Heteroptera

Ant-associations do not appear to be a ancestral condition in Hemiptera Such a result agrees with the fact that Formicidae is a recent taxon – the oldest known ant fossil is from Upper Cretaceous, 80 millions of years old (HÖLLDOBLER & WILSON, 1989, BOLTON, 1994) relatively to Hemiptera which are known since the Permian (see review in SORENSEN *et al.*, 1995) Ant-attendance occurred always after change of the habitat from on ground to above ground and subterranean associations with ants appear to be derived But not all above ground clades are ant-attended and there is no direct link (causation) between change of habitat and trophobiosis Probably changes of habitat to an above ground level occurred with changes of host plants One may expect that host plants mediate Hemiptera attractiveness and that some host plants are more suitable for trophobiosis than others

Source MNHN, Paris

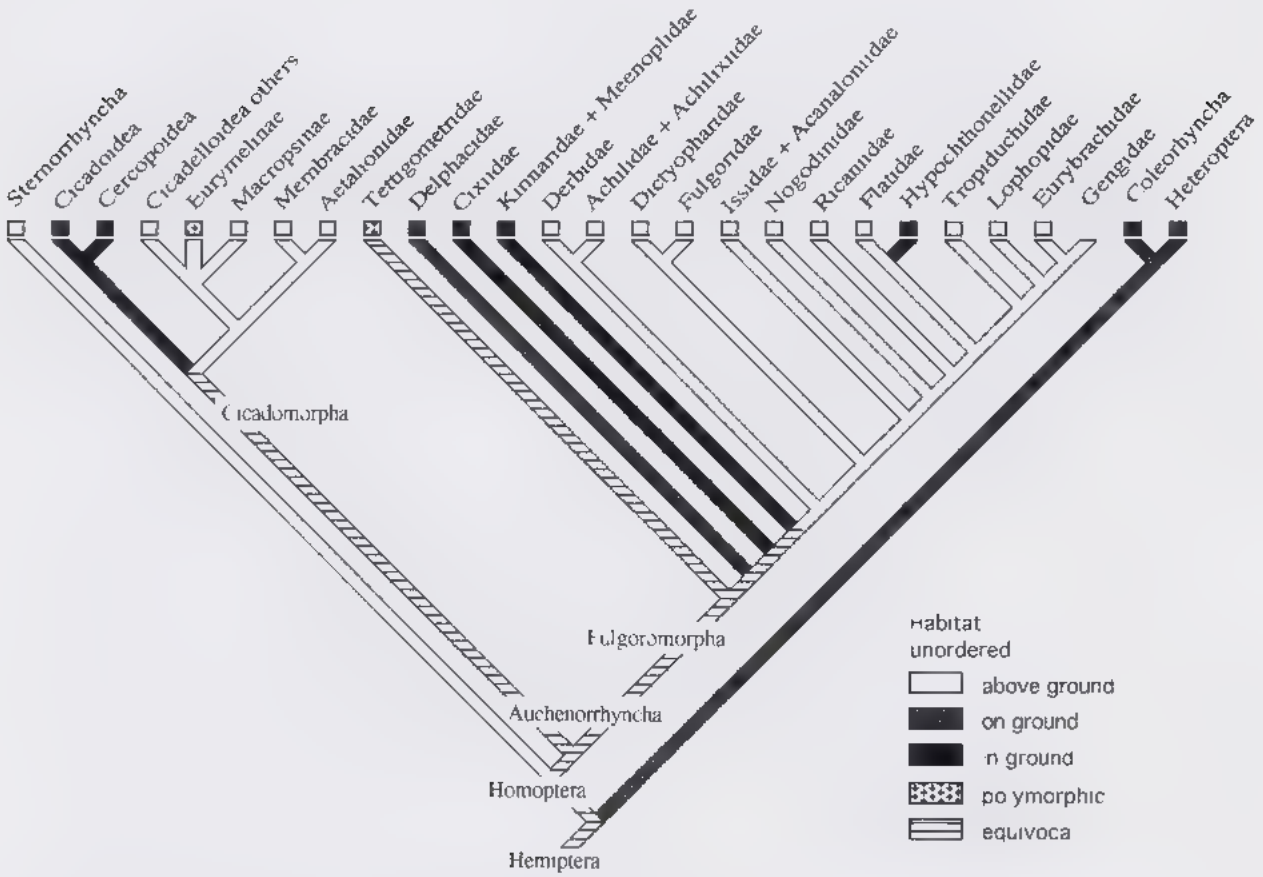

Fig. 4 - Optimization of the habitat attribute (in on or above ground) upon the phylogeny of the Hemiptera used by SCHAEFER (1987). One cannot choose between the character states for the Hemiptera, the Homoptera, the Auchenorrhyncha, the Cicadomorpha and the Fulgoromorpha.

In Fulgoromorpha, the cixiidian lineage retained the probable ancestral habitat on ground (at least at nymphs) and within this lineage, the Kinnaridae and several Cixiidae taxa went in a subterranean habitat (HOCH, 1994). From the paraphyletic Kinnaridae (BOURGOIN, 1993a) the above ground Meenoplidae family arose. In the sister lineage, the first Derbidae-Achilidae-Achilixiidae lineage specialized as fungal feeders (nymphs) under the bark of living and dead trees (WILSON *et al.*, 1994) while its above ground sister group radiated successfully in number (half of the known species in Fulgoromorpha) and in diversity (13 families). This success is probably related to angiosperm diversification but also with vicariance events linked to the breakup of Gondwana that led to the constrained distributions (absence in some biogeographical areas) observed in some of these families. Within the Fulgoromorpha and with exception of some scattered examples, the Tettigometridae is the only lineage which has developed strong mutualistic relationships with ants. One may expect that radiation of Tettigometridae from Tropiduchidae (BOURGOIN *et al.*, 1997) took place with change of host plants which have influenced their attractiveness to ants either directly (quality of honeydew) or indirectly (plant ant attractants).

Source: MNHN, Paris

Trophobiosis in Fulgoromorpha and the case of Tettigometridae. Prospectives

Theoretical studies (ROUGHGARDEN, 1975, WILSON, 1983, KEELER, 1985) suggest that mutualism should be restricted to situations where the cost of maintaining the situation is low to each participant, while the benefits are relatively great (BRISTOW, 1991) Fulgoromorpha-ant associations would seem to fit these restrictions Indeed, benefits/costs for both partners of such associations are already well known in other Homoptera and are also valid for Fulgoromorpha associations Ants benefit in reduced search time for food (honeydew) and for prey pursuit by direct predation on the hoppers, and in increased stability and quantity of the food resource Benefits for the fulgoromorphs include defense against predators which allows a relative perennial site of food and non accumulation of honeydew on the substrate (ROZARIO *et al*, 1993) Cost for ants includes active defense against predators and active monopolization of fulgoromorphan resources while a higher honeydew production by the hopper is requested

However among Fulgoromorpha, ant-mutualism is only documented in few species of Cixiidae (MYERS, 1929, THOMPSON, 1984), Delphacidae (DEJEAN *et al*, 1996), Issidae (DIETRICH & MCKAMEY, 1990), Hypochthonellidae (CHINA & FENNAH, 1952) and in most species of Tettigometridae (BOURGOIN, in prep) Why are so few Fulgoromorpha ant-attended? Why do more than 70% of the records concern the Tettigometridae? Are there phylogenetic constraints (morphological or behavioral adaptations) which could limit or favor ant-associations and should be hypothesized to account for this pattern? What is the impact of ant-attendance on such adaptations?

Although such generalizations should be approached with some caution (BRISTOW, 1991), regularly ant-attended aphids show several characteristics *e.g* cornicle length reduced, leg length reduced, not saltatorial, trophobiotic organ, (WAY, 1963, SKINNER, 1980, SUDD, 1987,) and placid behavior, gregariouness (DIXON, 1958, PIERCE *et al*, 1987) These characteristics seem to fit with those observed for the Tettigometridae within the Fulgoromorpha Indeed tettigometrid larvae share several unique morphological characteristics (no jumping apparatus, no wax plates, no sensory pits, a long anal tube, absence of anal combs and anal apodemes in adults) and particular behavioral traits (subsociality, sessile behavior)

Morphological characteristics Considering the function of these structures and correlation with myrmecophily, it appears legitimate to hypothesize an adaptive scenario that these autapomorphic characters in Tettigometridae have evolved under selection for tettigometrid-ant mutualism and thus may be secondarily simplified (versus showing a plesiomorphic state) or lost (versus primary absence) From a phylogenetic standpoint, all these tettigometrid characters have been considered plesiomorphic and homologous to the cicadomorphan state But, with a new careful and extended morphological analysis (thus independently with regard to this adaptive scenario to avoid circular reasoning), can the primary homology of these characters *a priori* hypothesized with the cicadomorphan state, be rejected? For instance, a new morphological analysis of the characters "long anal tube" and "absence of anal combs" have shown that they are in fact secondarily modified for the first, or reduced or secondarily absent for the second

Source MNHN Paris

Source MNHN, Paris

(BOURGOIN & CAMPBELL, 1996) At least, these two misinterpreted characters may carry an adaptive value for trophobiosis in ant-associations The other characters need to be reanalyzed according to this possibility

Sessile and subsociality behavior Four main types of ant-attendance have been reported in Fulgoromorpha literature 1 Opportunistic or occasional attendance by ants which collect on the substrate the honeydew drops randomly deposited by the planthopper kicking, such associations are generally limited in time (one flatid· PFEIFFER, 1996, some issids O'BRIEN, 1988, DIETRICH & MCKAMEY, 1990) 2 Underground attendance in ant nests (some cixiids MYERS, 1929, SHEPPARD *et al.*, 1979, THOMPSON *et al.*, 1979, THOMPSON, 1984, hypochthonellids (CHINA & FENNAH, 1952), some tettigometrids) 3 Attendance of planthoppers under shelters build by ants (some delphacids, reviewed in DEJEAN *et al.*, 1996) 4 Long time attendance, ants collects honeydew drops directly at the anal opening and regularly antennate the planthoppers (many tettigometrids, Figs 5-10)

DIETRICH & MCKAMEY (1990) have noted that all ant-attended Membracoidea and Cicadelloidea are sessile (non-jumping) and exhibit subsocial behaviors It is thus interesting to note that (i) all durable fulgoromorphan-ant associations are observed when planthoppers are either unable to escape (unground in ant galleries or under shelter) or sessile species (Tettigometridae) and (ii) all durable fulgoromorphan-ant associations are observed when planthoppers are either forced into gregariousness (cixiids kept in ant nests, delphacids kept under ant shelters) or when they are subsocial (tettigometrids) Such close correlations are noticeable and further studies are needed to elucidate the relationships between these different attributes ant-mutualistic, sessile, gregarious and subsocial behaviors

In Fulgoromorpha, modalities of trophobiosis appear to be quite diverse while it is limited to few species only Although morphological and behavioral characteristics seem important for mutualistic relationships between planthoppers and ants, one cannot forget that habitat and particularly host plants may have had an important impact on formation and maintenance of these associations This makes the Fulgoromorpha a nice model to study ant mutualism Mapping such attributes within a parsimonious evolutionary framework will allow to move from an anecdotal and descriptive natural history to a refutable evolutionary scenario of "how mutualistic interactions evolve and are maintained" (BRISTOW, 1991)

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FIGS 5-10 **5-9.** *Euphyonarthex phyllostoma* Schmidt (Fulgoromorpha Tettigometridae) attended by various species of ants (Hymenoptera, Formicidae) on *Bridelia micrantha* Baillon (Euphorbiaceae) in Cameroun **5:** Attended by *Camponotus brutus* Forel in the upper left part of the photograph one can also observe a dictyopharid (Fulgoromorpha Dictyopharidae) feeding. **6:** Attended by *Oecophylla longinoda* Latreille (in laboratory) **7.** Attended by *Camponotus acvapimensis* Mayr **8:** Attended by *Polyrhachis laboriosa* Smith (in laboratory) **9** Nymphs attended by *Myrmicaria opaciventris* Emery **10:** *Hilda rubrospersa* Fennah (Fulgoromorpha Tettigometridae attended by *Camponotus sp* (*brutus* group) (Hymenoptera, Formicinae, on *Ficus vallis choudae* Delile (Moraceae), in Cameroun Photographs by A DEJEAN

Source MNHN, Paris

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Source MNHN, Paris

Food Choice and Environment Occupancy in Afrotropical Dung Beetles: a Phylogenetic Study of two Examples (Coleoptera, Scarabaeidae)

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ABSTRACT

Phylogenetic studies of two genera of Afrotropical Scarabaeidae dung beetles (*Euonticellus* and *Milichus*) have enabled to clarify some points in their evolutionary history. The species of *Euonticellus* which have experienced an early separation from the rest of the genus live in forest and use non-ruminant mammals dung of the elephant type. Phylogenetic analyses enable to assume that the ancestor had the same macrohabitat and microhabitat. In the course of evolution, the genus seems to have gradually invaded savanna environments and ruminant mammals dung, when these new habitats were available. These changes considerably enlarged the genus' ecological niche. The genus *Milichus* experienced the same change in food, but the change in environment has been inverse and took place from savanna to forest.

RÉSUMÉ

Régime alimentaire et utilisation de l'environnement chez des coléoptères coprophages afrotropicaux : étude phylogénétique de deux exemples (Coleoptera, Scarabaeidae)

L'étude phylogénétique de deux genres de Scarabaeidae coprophages afrotropicaux (*Euonticellus* et *Milichus*) a permis de préciser certains éléments de leur histoire évolutive. Pour ce qui est des *Euonticellus*, les espèces qui se sont séparées précocement du reste du genre vivent dans des milieux de forêt et exploitent les déjections de mammifères non ruminants, du type de l'éléphant. La même analyse permet de penser que l'ancêtre du genre avait les mêmes macrohabitat et microhabitat. Dans le courant de l'évolution, le genre semble avoir envahi les milieux de savane et les excréments des mammifères ruminants au fur et à mesure que ces nouveaux habitats étaient disponibles. Ces changements ont considérablement agrandi la niche écologique du genre *Euonticellus*. Le genre *Milichus* a connu le même changement de nourriture, mais son changement d'environnement s'est fait en sens inverse : de la savane vers la forêt.

INTRODUCTION

The present environments of tropical Africa can be divided into two well-defined and contrasted groups : tropical evergreen rainforest and both grasslands and woodlands, these two latter environments or biomes constituting the "savanna" category (especially in West Africa

CAMBEFORT, Y., 1997 — Food choice and environment occupancy in Afrotropical dung beetles : a phylogenetic study of two examples (Coleoptera, Scarabaeidae). *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects : Phylogenetic Tests of Evolutionary Scenarios. *Mém. Mus. natn. Hist. nat.*, **173** : 125-134. Paris. ISBN : 2-85653-508-9.

Source : MNHN, Paris

GHAZANFAR, 1989) Most Afrotropical plants and animals live in either forest or savanna This division is clear-cut, at least as far as Scarabaeidae dung beetles are concerned no forest species inhabit savanna, and vice versa (CAMBEFORT, 1991c, CAMBEFORT & WALTER, 1991) However, most of the generally recognized genera (*i.e.* monophyletic ensembles of species – as far as phylogenetic studies have established their monophyly) comprise forest and savanna species Therefore, in these monophyletic groups, some species must have changed from one environment to another In each case, it can be asked which was the ancestral environment, and what was the influence of this change on the history of the genus

The vegetal environment constitutes what could be called the "macrohabitat" of populations Within these macrohabitats, the Scarabaeoidea lineage experienced changes in diet from a supposed mycophagy to such advanced diets as wood, flowers and dung (SCHOLTZ & CHOWN, 1995) So-called dung beetles live in and around the "microhabitats" made up by the excrements of animals, especially of mammals Dung beetles also use these excrements as their food Although these insects are able to accept different sorts of dung, they seem to have rather precise requirements for adult food, and especially for the making of brood balls which larvae eat from inside and develop in (CAMBEFORT & HANSKI, 1991) According to these preferences, it is possible to divide dung beetles into omnivore dung specialists and herbivore dung specialists In this latter category, it is possible to distinguish between non-ruminant herbivore dung specialists and ruminant herbivore dung specialists (CAMBEFORT, 1984, 1991c, TRIBE, 1976) In the second case, most genera comprise both categories of specialists In the course of each genus' history, changes in food (microhabitat) can have occurred, as well as in macrohabitat Therefore, it can be asked how these changes have occurred, and what was their influence on the evolutionary history of the genus To test this two-fold problem (changes in macro- and microhabitat), two genera of dung beetles have been selected *Euonticellus* and *Milichus* Both are specialists of large mammals' dung But some of their species occur in forest, some others in savanna, some species prefer the dung of Bovine mammals (including cattle), some others are found only in elephant dung

MATERIAL AND METHODS

The taxa

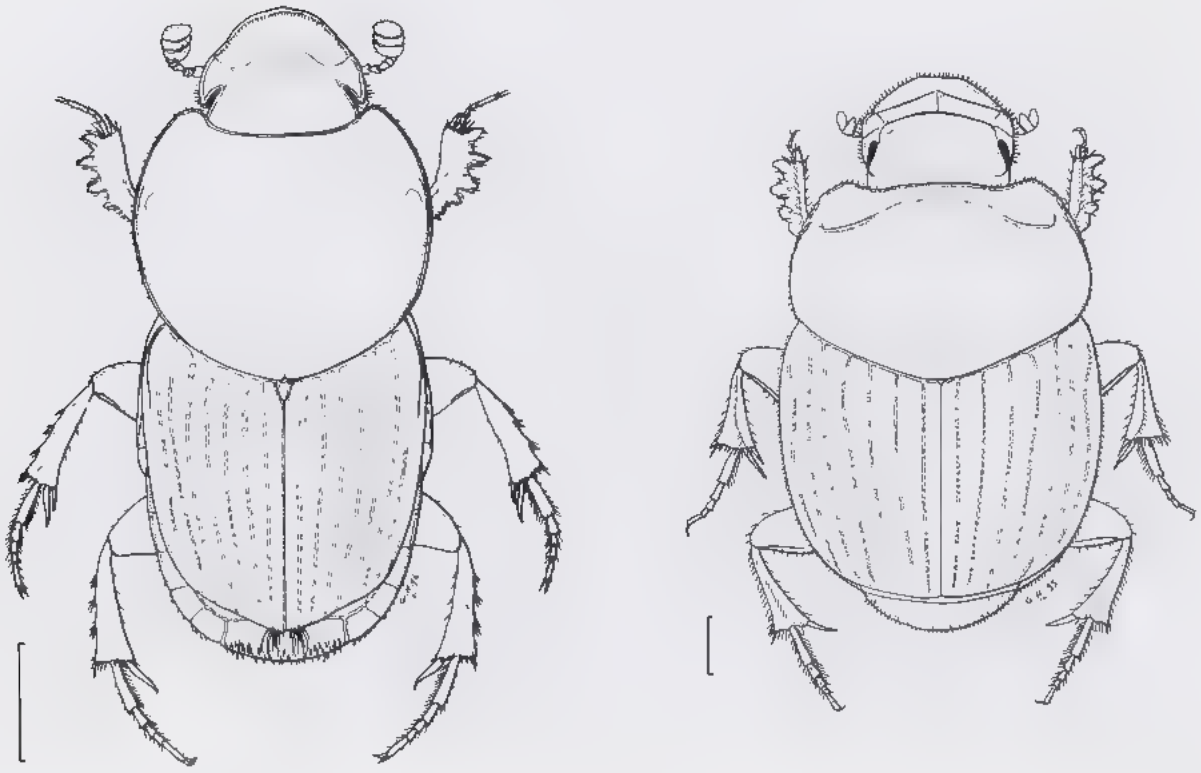
Genus *Euonticellus* Janssens 1954 The 19 species of the genus (habitus Fig 1) occur mostly in tropical Africa (14 species, There are 2 species in the Palaearctic region, 2 in the Oriental region (including one in common with the Palaearctic region), and one Neotropical species, restricted to Cuba and Jamaica These species are specialists of large herbivore mammals' dung, especially elephant and Bovini (including cattle) Some species are among the most evolved and efficient dung beetles, because they have developed optimal use of dung Their larvae use the most nutritious part of this dung ("coprobiontic" alimentation CAMBEFORT, 1991a), which allows the female to lay eggs a very short time after emergence (down to 5 days HALFFTER & EDMONDS, 1982, A phylogenetic study of the genus, which will not be detailed here, has produced the cladogram of Figs 3-4 (CAMBEFORT, 1996b)

Genus *Milichus* Péringuey, 1901 The taxon (habitus Fig 2) is endemic of Afrotropical region There are 15 described species, all specialists of herbivore mammals' dung, which live both in savanna and forest As for *Euonticellus*, a phylogenetic analysis of this genus has recently been published (CAMBEFORT, 1996a)

Habitat choice

Habitat (both macro- and microhabitat choices are here considered as "attributes" of the relevant species (see following paragraph) These choices of most of the species of the genera *Euonticellus* and *Milichus* have been established from field works, especially the author's ones (published or not), and according to other authors (*e g* KINGSTON 1977) As for the environment (macrohabitat), there is no possible doubt the species clearly occur either in forest or in savanna (grassland and/or woodland) Food (microhabitat) choice is sometimes less clear-cut It has been considered that, when more than 90 %

Source MNHN, Paris



FIGS 1-2 *Euoniticellus* and *Milichus* habitus 1. *E. capnus* Cambefort 1996 2: *M. boucomonti* Cambefort 1996 (Scale bars = 1 mm)

of the individuals occurred in either of the categories taken into account (elephant dung or cattle dung) the relevant species was a specialist of this sort of excrement (HANSKI & CAMBEFORT, 1991) When there was some doubt, the choice (the attribute) has been considered as polytypic

Evolution of attributes

The ecological traits have not been used for establishment of the relevant phylogenies These traits are not considered as characters but rather as attributes, according to the definition given by GRANDCOLAS *et al.* (1994). "an attribute is a trait of which primary homology is questionable because it does not match the homology criteria, an attribute is empirically an extrinsic or widely defined trait, it is not used for phylogeny construction but is studied in reference to an independent phylogeny" For this reason, some consensus exists in favor of treating ecological aspects of the niche as attributes and not to use them to construct the tree whose aim is to clarify their changes (*e.g.* BROOKS & MACLENNAN, 1991)

For the present study, the program MacClade, version 3.04 was used This program has a set of functions which enable the study of the analysis of characters, including traits not used to construct the tree (MADDISON & MADDISON, 1992, 1993) These functions use the classical principles of character optimization according to Fitch parsimony (*e.g.* KITCHING, 1992)

RESULTS

Genus Euoniticellus

The phylogenetic study of the genus (CAMBEFORT, 1996b) shows that it can be divided into two clusters of species a paraphyletic cluster (from *perniger* to *parvus*), which comprises smaller species (average length 5 mm), a monophyletic group (from *cubiensis* to *pallipes*),

Source MNHN, Paris

which comprises species whose average length is almost two times bigger (9 6 mm) The former cluster consists of species close to the tree root, and which, in this hypothesis of phylogeny, can be considered for this reason as "older" They occur mostly in tropical Africa, with one species in tropical Asia In general, they are rare or very rare, with restricted geographical distribution, and most of them have been described rather recently The latter group represent a monophyletic clade which consist of probably more recent species They are more widespread than the former species, with a vast geographical distribution Most of them have been described a longer time ago (XIXth or even XVIIIth century) Most of them occur in Tropical Africa, but some also occur in Palaearctic and/or Oriental regions, with one Neotropical species These 11 species are the descendants of one ancestral species and constitute the sister-group of one species of the former group *E. parvus* The ancestral species split from the stem of the "older" species at some time during the evolution of the genus, and experienced an especially important cladogenesis which gave rise to a clade of eleven species

On the relevant phylogeny, the ecological attributes have been mapped food or microhabitat and environment or macrohabitat There is a very clear difference between smaller and larger species as far as food is concerned (Fig 3) while the former prefer elephant dung (only *E. parvus* occurs exclusively in cattle), most of the latter occur in cattle dung (only the twin species *E. kawanus-tibatensis* prefer elephant dung) The difference is also clear in the case of the environment (Fig 4) most of the smaller, elephant specialist, basal species occur in forest, most of the larger, cattle specialist, apical species occur in savannas

Genus Milichus

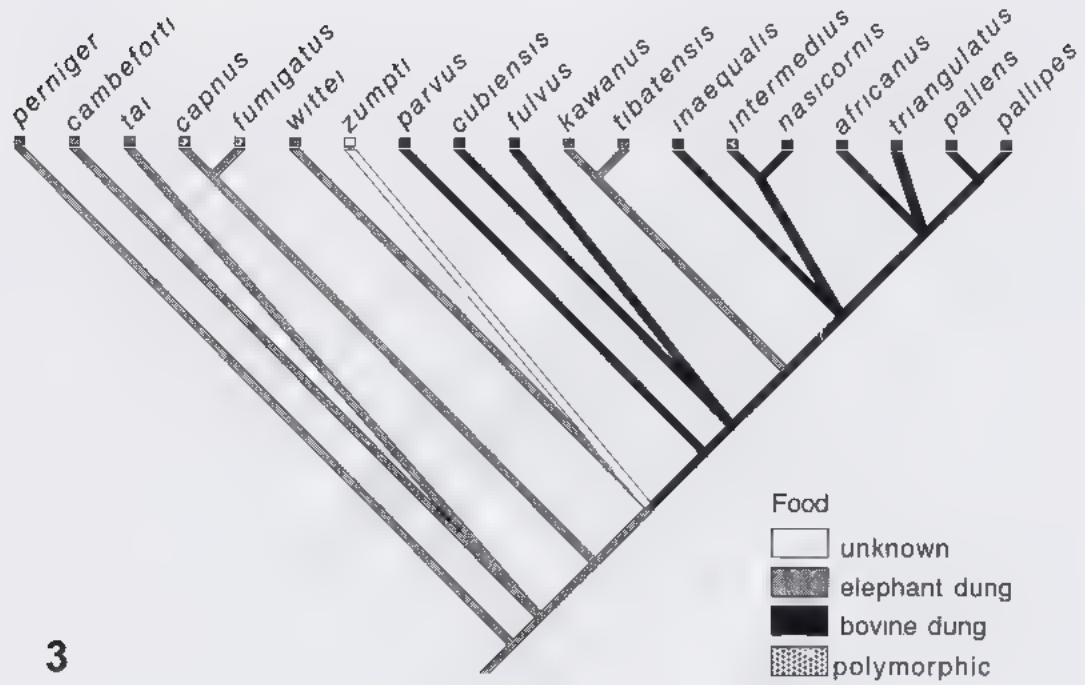
The phylogeny of this genus (CAMBEFORT, 1996a) does not show such clear-cut species groups as in the preceding case Only the 4 species of the top of the tree, which form a monophyletic group (with two pairs *dudleyae/dudleyi* and *inaequalis/lecourti*) are of a smaller size than the other species In the same way as *Euonticellus*, two ecological attributes have been studied food and environment As for food choice (Fig 5), although it is unknown for 4 species out of 15, it seems clear that a mere minority of the species occurs in cattle dung (also 4 species out of 15) On the contrary, elephant dung seems to be the choice of 8 species On the 15 described species, 10 live in herbaceous environments, grasslands and/or woodlands (Fig 6) A mere 5 species occur in evergreen rainforest, of which 4 constitute a monophyletic group, and the 5th one seems to have diversified separately and specialized for this environment from a step living in humid savanna of the Guineo-Congolian type (GHAZANFAR, 1989)

DISCUSSION

The ecological attributes which have been reported on the phylogenetic hypotheses of the genera *Euonticellus* and *Milichus* are recent The past environments were probably not the same as the present ones Also, it is difficult to know which sort of dung was used as food by the

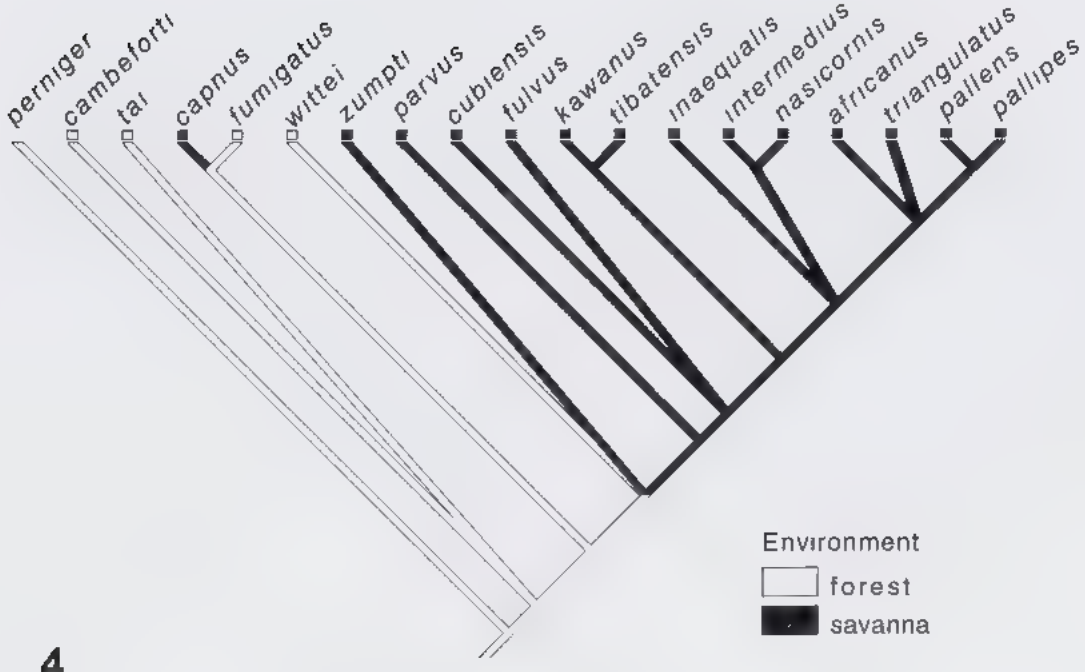
FIGS 3-4 — Phylogeny of the genus *Euonticellus*, with ecological attributes optimized **3**: food (microhabitat), **4**: environment (macrohabitat).

Source MNHN Paris



3

Euoniticellus : food



4

Euoniticellus : environment

Source MNHN, Paris

ancestors of the living species But I assume that, in the early history of the genus, both macro- and microhabitats were not very different from the present ones In the phylogenetic tree of *Euonticellus*, ecological attributes show a clear-cut change both in food and environment Smaller, "primitive" species live(d) in forest environments and use(d) the dung of browser mammals of the type of the elephant's These are coarse dung types, small parts of which only can be used by smaller dung beetles (TRIBE, 1976) At some time in the genus' history, one of these species seems to have changed this diet for some grazer mammal dung, probably a ruminant one The question is open as to the time – in the genus' evolution – when this change took place In the present species, there is a "small" one (*E. parvus*) which feeds on cattle dung On the other hand, there is a pair of "large" species (*E. kawanus-tibatensis*) feeding on elephant dung According to MacClade's optimization (Fig 3), this change occurred before the differentiation of *E. parvus*, and the specialization of the pair *kawanus-tibatensis* is of secondary nature In any case, this change was a very important innovation, which opened quite a new area (or "niche") to the genus, and made possible the cladogenesis which produced the more modern species of the genus It is possible that this cladogenesis was an adaptive radiation, which took place to fill the new niche of grazer mammals' dung

These grazers, perhaps ruminants, no longer lived in the forest Food change of *Euonticellus* coincided with environment change part of the forest which has originally covered almost the whole tropical Africa, was gradually changed into savanna woodlands in the more humid areas, grasslands in dryer ones This new environment, rich in grasses of the family Graminaceae (Poaceae), induced the evolution of quite a new fauna of grazer mammals, among which the Bovini possess the most evolved digestive adaptations Their dung has a very fine structure and allows an optimal use by dung beetles, both adults and larvae The joint change in micro- and macrohabitat, which possibly occurred almost at the same time (at the geological scale), opened a new niche ruminant-dung-in-savanna, which has been exploited by the *Euonticellus* species ancestor of the group *cubiensis-pallipes* This new niche has proven more successful than the older one (browser-dung-in-forest), enabling the adaptive radiation of modern *Euonticellus*, which have occupied all the environments of savanna, and even gone out the tropical areas, probably following their mammalian sources of food Moreover, cattle domestication by man even enlarged this niche and geographical distribution of the genus Up to this point, the spreading of the taxon has been a natural one Recently, the efficiency of some *Euonticellus* in recycling cattle waste, having been taken into consideration, one species (*E intermedius*) has been introduced artificially into Australia It is now one of the most successful of the introduced species in this area (DOUBE *et al* , 1991) The species has also been introduced into other Oceanian areas New Caledonia and Vanuatu (GUTTIFRREZ *et al.*, 1988), and also in (sub)tropical America (FINCHER, 1986)

Coming back to the adaptive radiation of the modern species of *Euonticellus*, it is worth remarking that their size is larger than for those using elephant dung This is in contradiction with a previously established "rule" (*e.g.* CAMBEFORT, 1991c), according to which there is a

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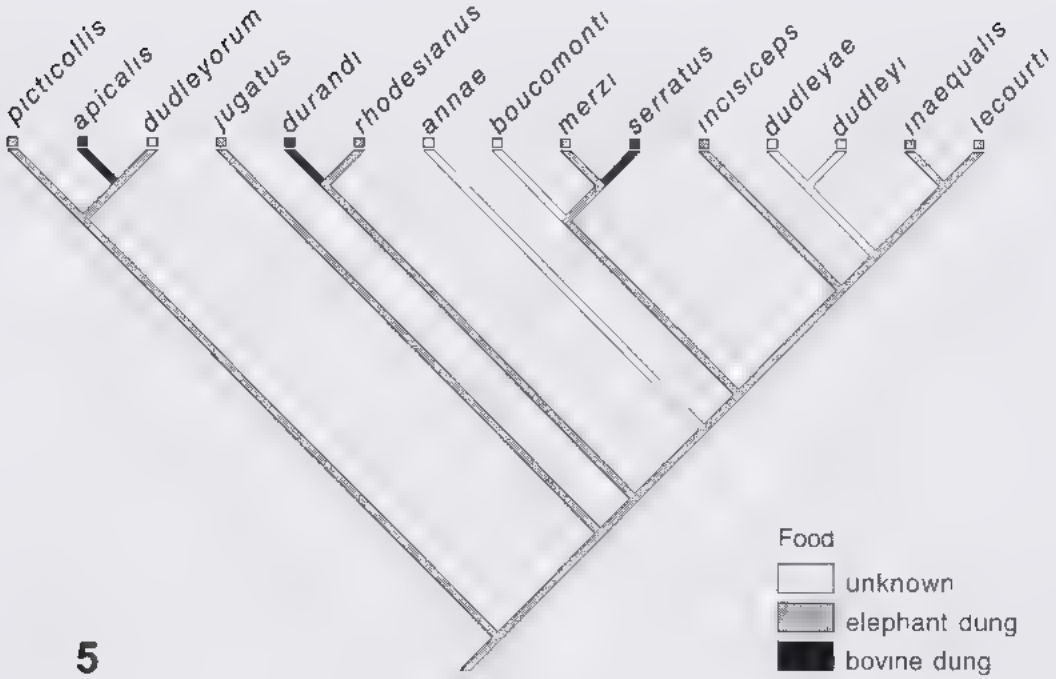
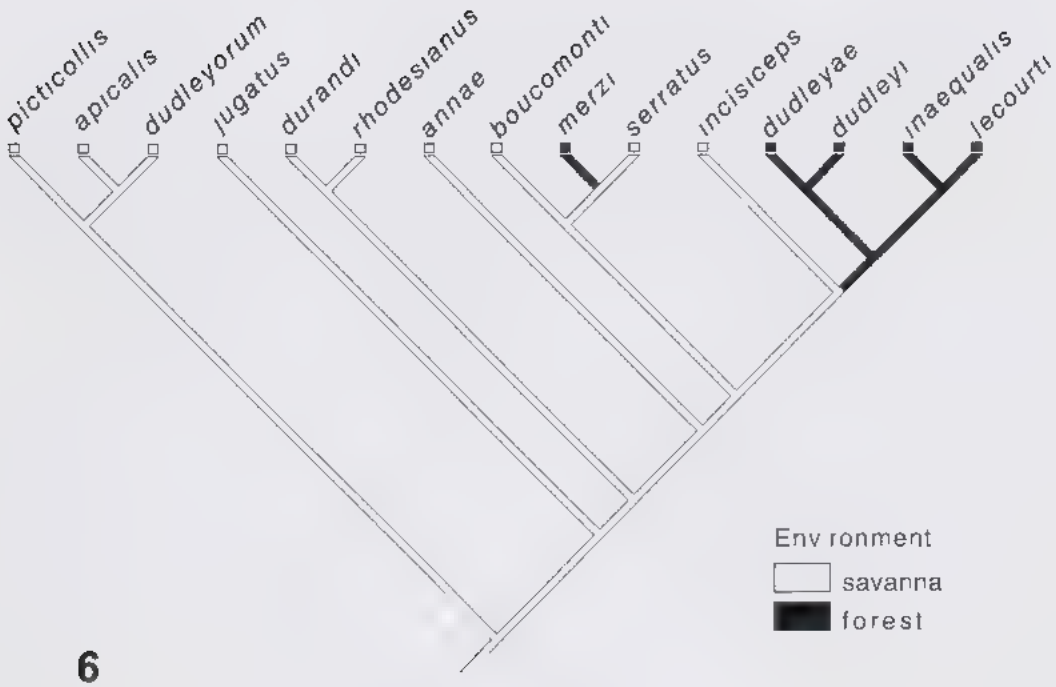
FIGS 5-6 Phylogeny of the genus *Mithchus*, with ecological attributes optimized **5.** food (microhabitat), **6.** environment (macrohabitat)

Source MNHN, Paris



Milichus : food



Milichus : environment

Source MNHN, Paris

correlation between average size of mammals and of dung beetles using their dung Now, this is a general rule, valid for the entire dung beetle fauna In the case of the genus *Euonticellus*, optimal utilization of cattle dung, which is highly characteristic of the genus, and higher alimentary value of this type of dung, possibly enabled the species having this diet to reach a larger average size than those exploiting elephant dung On the contrary, size ratio in the genus agrees with another "rule" according to which savanna dung beetle species have an average size larger than forest species (CAMBEFORT & WALTER, 1991).

Is it possible to give a minimum date, even approximate, of the change of habitats that lead to the "modern" *Euonticellus*? First pollen grains of grass appear, in Africa, in the mid-Eocene (VAN DER HAMMEN, 1983), documenting the first grassland and woodland areas During the Cenozoic, grass formations evolved between forests and (sub)deserts, probably at the expense of the former, and at least to some extent owing to the action of "megaherbivores" (OWEN-SMITH, 1988), *i.e* large herbivorous mammals of the elephant type, which seem to have been abundant in Africa at least from Miocene to Holocene (KALB, 1995) Due to their action, arboreal vegetation disappeared on vast expanses of land, where grass was able to develop (CAMBEFORT, 1991b, 1991c) But it was for the benefit of other mammals the so-called grazers, including ruminants, group of which the Bovini represent the more advanced branch It is in the Pliocene that recent Bovini began to appear in Africa, probably from Asia (*e.g.* GENTRY, 1990, 1992, GERAADS, 1992, THOMAS, 1984, VRBA, 1985) It is from that time on that modern *Euonticellus*, which may have begun to settle in woodland areas in large mammal dung (*cf.* the *E. kawanus-tibatensis* pair), must have started to use Bovini dung More Bovini gained importance and number, more modern *Euonticellus* became widespread, in Africa first, then in Asia, and finally in America, of which *E. cubiensis* is up to now the proof Today, this species lives in grassland areas in Cuba and Jamaica, where it uses cattle pats (MATTHEWS, 1966) It is possible that its ancestors once followed Bovini troops during their migration eastward As Bovini have never reached the West Indies, we must assume that this dung beetle has, for some reason, changed its diet and started to use the dung of some large American mammals Edentata (Xenarthra) of the group of terrestrial sloths (Megalonychidae), for these were the only large mammals in Cuba (ITURRALDE-VINENT, 1988) The beetle then might have followed the mammals in their land or sea journey towards Large Antilles Finally, the mammals disappeared there, and the beetle turned back to Bovini dung, when cattle was introduced into the islands, from XVIth century on This is a rather complicated history, but the real "scenario" may have been even more complicated In any case, the species is not very different from other "modern" *Euonticellus*, and does not make a particular section in the genus (contrary to MATTHEWS, 1966)

All the precedent paragraphs dealt with *Euonticellus* If we now consider the genus *Milichus*, and first its microhabitat (food), it seems that there has been also a change from elephant ("old" species diet) to Bovini dung ("modern" species diet). In this case, change of macrohabitat seems to have been from savanna to forest Africa occupancy by *Milichus* species seems to have been not "centrifugal" (*i.e.* from central forest to peripheral savannas, as in *Euonticellus*) but centripetal (*i.e.* from savanna to forest) Now, centrifugal dispersion (in Africa) leads to a larger distribution than a centripetal one, due to the fact that the expanse of the savannas are larger than that of the forest, and used to be even larger because the relative extension of the forest versus savanna is now larger than average (MALEY, 1996) Older species of *Milichus* seem larger than more modern ones These size relationships are less clear than in

Source MNHN, Paris

the precedent genus Moreover, if the spreading really took place from savanna to forest, it is difficult to date it

CONCLUSION

Study of evolution of two ecological attributes food (microhabitat) and environment (macrohabitat) in two genera of Afrotropical dung beetles enables one to formulate some hypotheses concerning "evolutionary scenarios" of these genera The shift from use of non-ruminant dung to ruminant (especially bovine) dung is assumed in the two cases in question On the other hand, the shift from forest to savanna seems to have taken place in one case out of two In any case, it is clear that those species whose evolution is in conformity with the double scenario "non-ruminant dung → ruminant dung", and "forest → savanna" will be promoted by all means, including human action This last factor in turn can act at two levels

- passive action destruction of Afrotropical forest and of elephant, multiplication and dissemination of domestic cattle,
- active action introduction of dung beetle species into areas where they do not occur

This is the case of some "modern" *Euonticellus*, particularly of *E intermedius*, which is now one of the most abundant and widespread dung beetle on earth On the contrary, older species are "trapped" both in their macrohabitat and microhabitats Coming back to the word "scenario", and giving it its proper meaning of "history", it could be said that the older species are not (or no longer) "in the sense of History" The scenario can even be expanded in the future, and it can be predicted – in the true meaning of the word, *i.e.* in the future – that the older species will get extinct before the more modern ones, and lamentably perhaps in a short span of time, together with the Afrotropical forest and elephant

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Source: MNHN, Paris

Phylogenetic Relationships among European *Polistes* and the Evolution of Social Parasitism (Hymenoptera: Vespidae, Polistinae)

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ABSTRACT

Cladistic analysis of the European species of *Polistes* is used to investigate the evolution of social parasitism in the genus The three species of social parasites (formerly the genus *Sulcopolistes*) are all inquilines lacking a worker caste, and dependent on usurping the colony of a host species to obtain a worker force EMERY's Rule states that social parasites are more closely related to their hosts than to any other species Previously published allozyme (CARPENTER *et al*., 1993, and mtDNA (CHOUDHARY *et al*., 1994) data did not support this hypothesis, but did not resolve relationships among the species of social parasites Morphological characters are adduced which resolve the phylogenetic relationships among these three species, and the combination of the morphological and molecular data sets largely resolves the relationships among all nine European species Cladistic optimization of traits associated with social parasitism on the resulting cladogram shows (1) EMERY's Rule is rejected, (2) the scenario proposed by TAYLOR (1939) for the evolution of social parasitism is not supported either The predatory behavior of the inquiline *P atrimandibularis*, with separate "supply" and "nursery" nests is evidently secondary, as is its initially passive invasion behavior

RÉSUMÉ

La phylogenie des *Polistes* d'Europe et l'évolution du parasitisme social (Hymenoptera : Vespidae, Polistinae)

L'analyse cladistique des espèces de *Polistes* européennes est utilisée pour étudier l'évolution du parasitisme social dans le genre Les trois espèces de parasites sociaux (autrefois le genre *Sulcopolistes*) sont toutes « inquilines » elles n'ont pas de caste ouvrière et s'approprient celle de la colonie d'une espèce hôte Le règle d'EMERY affirme que les espèces de parasites sociaux sont plus proches parents de leurs hôtes que de toute autre espèce Des analyses basées sur les allozymes (CARPENTER *et al*., 1993) et l'ADN mitochondrial (CHOUDHARY *et al* 1994) n'ont pas confirmé cette hypothèse, mais n'ont pas résolu non plus les relations entre les espèces parasites L'addition de caractères morphologiques et la combinaison des données morphologiques et moléculaires ont permis de résoudre respectivement les relations phylogénétiques entre ces trois espèces, et entre les neuf espèces européennes L'optimisation sur le cladogramme de traits associés au parasitisme social montre que (1) le règle d'EMERY est réfutée, (2) le scénario d'évolution du parasitisme social proposé par TAYLOR (1939) est également réfuté Le comportement prédateur de l'inquiline *P atrimandibularis*, qui maintient des nids séparés pour les provisions et pour le couvain, est à l'évidence secondaire, de même que son comportement d'invasion initiale passive

CARPENTER, J M 1997 — Phylogenetic relationships among european *Polistes* and the evolution of social parasitism (Hymenoptera Vespidae, Polistinae) *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects Phylogenetic Tests of Evolutionary Scenarios *Mém Mus nam Hist nat* **173** 135 161 Paris ISBN 2-85653-508-9

Source MNHN Paris

INTRODUCTION

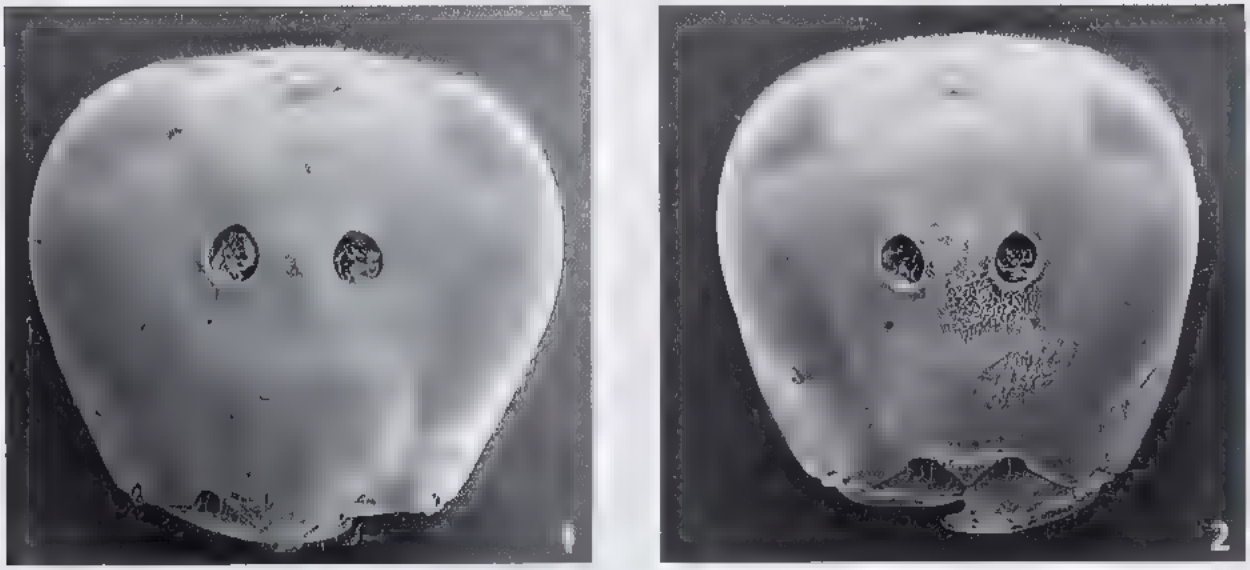
The social parasites known as inquilines are among the most intriguing outcomes of evolution in paper wasps Inquilines have no worker caste and cannot build nests, they must invade the colony of a host species and supplant the queen to obtain workers to rear their brood Striking morphological differences occur between host and parasite (*cf.* Figs 1-2) This form of behavior is rare in paper wasps, having been found in just three species in one genus, *Polistes* *Polistes* is a cosmopolitan genus, with more than 200 described species (CARPENTER, 1996b), the three social parasites (*atrimandibularis*, *semenowi* and *sulcifer*) are found only in Europe and the Mediterranean Region The inquiline behavior has long been known (WEYRAUCH, 1937), but recent years have seen a profusion of studies, particularly by workers at the University of Florence (for a review see CERVO & DANI, 1996) These studies have revealed remarkable phenomena, such as simultaneous domination by *atrimandibularis* of several colonies of the host *biglumis*, only one of which serves for parasite brood rearing while the larvae of the other nests serve as a source of food (CERVO *et al.*, 1990c), or change in the composition of the chemical signature of *atrimandibularis* to match that of *biglumis* at the time of emergence of host workers (BAGNERES *et al.*, 1996) This wealth of new information has stimulated intense interest in evolutionary explanations of aspects of social parasitism

In this paper I apply cladistic analysis to investigate the evolution of social parasitism in *Polistes* I first review evolutionary explanations that have been advanced for the social parasites, and how cladistic tests may be constructed for such hypotheses I then present the first cladistic analysis of interrelationships among the inquilines and related species to be based on morphology These characters are also combined with previously published molecular data, and analyzed simultaneously Behavioral features associated with social parasitism are optimized on the resulting cladogram, to test various evolutionary hypotheses

EMERY's Rule

The classical explanation that applies to the origin of socially parasitic species is the hypothesis known as EMERY's Rule (after EMERY, 1909) EMERY's Rule is that social parasites are more closely related to their host species than to any other species This is usually interpreted to mean that social parasites evolved directly from their hosts, either by sympatric speciation (*e.g.* WEST-EBERHARD, 1986, BUSCHINGER, 1986, 1990, BOURKE & FRANKS, 1991) or not (*e g.* WILSON, 1971) Alternatively, social parasites may exploit similar recognition systems in closely related species (CARLIN, 1988) However, previous cladistic analyses do not support EMERY's Rule in *Polistes* CARPENTER *et al.* (1993) presented an allozyme data set for the three species of social parasites, their four host species (see Table 1) and two outgroup species The 27 loci were subjected to three different coding procedures, which led to different results for each method, but under none of the codings were the social parasites most closely related to their hosts CHOUDHARY *et al.* (1994) analyzed a 386 base pair sequence from the mitochondrial 16S rRNA gene Their cladistic results were less ambiguous, with two cladograms, differing only in the interrelationships among the social parasites The social parasites were a monophyletic group Thus, phylogenetic analysis of two independent sources of evidence, by rejecting close relationship of parasite to host, rejected EMERY's Rule

Source MNHN, Paris



FIGS 1-2 — Frontal view **1**: *Polistes dominulus*, **2**: *Polistes atrimandibularis*. The magnification is 21x

TABLE 1 — Host-parasite relations among European *Polistes* species. CERVO & DANI (1996) report experimental introduction of *atrimandibularis* into the nests of *nimphus*, but this is unknown to occur naturally

Parasite	Host
atrimandibularis	*biglumis, gallicus*
semenowi	*dominulus, nimphus*
sulcifer	*dominulus*

TAYLOR's Scenario

EMERY's Rule also played a role in development of an evolutionary scenario for the origin and elaboration of social parasitism in wasps, that of TAYLOR (1939). TAYLOR observed a case of nest usurpation of the vespine species *Vespula vidua* by *V. squamosa*, then considered closely related. He then suggested a sequence of behavioral changes from free living to inquiline. The scenario consisted of four stages: (1) Intraspecific, facultative, temporary parasitism, (2) Interspecific, facultative, temporary parasitism, (3) Interspecific, obligate, temporary parasitism, (4) Interspecific, obligate, permanent parasitism.

Intraspecific nest usurpation thus evolved into inquiline behavior, with the loss of a worker force produced by the usurping queen. The existence of intraspecific nest usurpation was well known in vespines (JANET, 1903), as were inquilines (*e.g.* WEYRAUCH, 1937), and TAYLOR's scenario became generally accepted (*e.g.* WILSON, 1971; MATTHEWS, 1982; GREENE, 1991). The applicability of this hypothesis to polistine social parasites has been questioned by CARPENTER *et*

Source: MNHN, Paris

al. (1993) and CHOUDHARY *et al.* (1994), who observed that whereas the first stage in TAYLOR's scenario was common in paper wasps, the second stage was rarely reported, and the third stage is unknown (see review in CERVO & DANI, 1996) Nevertheless, the general notion of evolution of usurpation into inquilinism was accepted

Recent hypotheses

The recent increase in knowledge of the behavioral ecology of the inquilines has been accompanied by novel hypotheses on the evolution of features of their biology, some newly reported Notable examples discussed by CERVO & DANI (1996) include observations on the distribution of parasites and length of the colony cycle in one host, timing of usurpation by the parasite, the tactics employed by the parasite in dominating the host queen, and the predatory behavior of *atrimandibularis* These ideas are briefly summarized here

The Florence group has shown that inquilines are moderately abundant at the foot of mountain ranges The hosts nest mostly at low elevations, but *biglumis* nests high in the mountains (800-2000+ m in Italy, see CERVO *et al.*, 1990b) Obligate parasitism is suggested to have evolved from such a high altitude species (LORENZI & TURILLAZZI, 1986), because the colony cycle is short (four months, *vs.* six in the low altitude hosts), which is correlated with a high frequency of intraspecific usurpation Synchronization of colony cycle, resulting in availability of suitable host nests (WCISLO, 1987), may also explain the limitation of inquiline behavior to temperate regions Migratory behavior (between lowlands and mountain tops in both inquilines and hosts in Italy) may be related to the origin of obligate parasitism, whether through decreased kinship (CERVO & DANI, 1996) or through reduction in population size leading to enhanced parasitic tactics (WEST-EBERHARD, 1996).

Local nesting environment may also influence timing of invasion by the inquiline Colonies at higher altitudes are invaded earlier in the colony cycle, in the middle of the period before worker emergence (pre-emergence phase), while those in the lowlands may be invaded early in the period after workers have begun to emerge (post-emergence phase) Inquilines tend to prefer larger nests in more advanced stages of development among available nests (CERVO *et al.*, 1993) Early invasion by *atrimandibularis* in *biglumis* nests may be an adaptation to the shortened colony cycle of the host, because usurpation while workers are emerging would entail delay in production of inquiline offspring, jeopardizing reproductive success at high altitudes (CERVO *et al.*, 1990c)

The inquilines differ in tactics employed during usurpation In *sulcifer*, aggressive fighting generally leads to the expulsion or death of the host queen (SCHEVEN, 1958, DISTEFANO, 1969, TURILLAZZI *et al* , 1990) Aggressive tactics, when invading hosts at low altitudes, may occur because the inquiline can afford to dispose of the host queen, and retain only individuals that contribute to the worker force (CERVO *et al.*, 1990b) The tactics of *semenowi* are also usually aggressive (SCHEVEN, 1958, CERVO *et al.*, 1990b, MEAD, 1991, ZACCHI *et al* , 1996), but may be passive, with the host queen remaining on the nest (DEMOLIN & MARTIN, 1980) The tactics of *atrimandibularis* are variable Initially it is passive, temporarily submissive when invading *biglumis* (CERVO *et al* , 1990a), and the host queen of *biglumis* remains on the nest When invading *gallicus* nests the host queen is expelled (CERVO *et al* , 1992) But when invading colonies of *biglumis* later in the season *atrimandibularis* employs violent, fighting tactics (SCHEVEN, 1958) It also is more aggressive on the secondary nests that serve as food supply

Source MNHN, Paris

than on the primary nest (nursery nest) where brood is reared (CERVO *et al*., 1990a) The tactical flexibility of *atrimandibularis* is thus correlated with characteristics of the invaded nest, such as number of foundresses present, length of colony cycle, and the point in the colony cycle where invasion occurs (CERVO *et al.*, 1990b, CERVO & DANI, 1996) The passive tactics of this inquiline may be considered unexpected in a generalist (*i.e.*, with several hosts; FISHER, 1984), but may be explained if *biglumis* is the original host of *atrimandibularis*, for which *atrimandibularis* has evolved specialized tactics (CERVO & DANI, 1996) Cohabitation of the inquiline and host queen may indicate an advanced form of parasitism, for example involving chemical control, or it may be a necessity if the inquiline is unable to inhibit ovarian development of host workers (CERVO & LORENZI, 1994), and so would be a less advanced type of parasitism

The outstanding feature of the behavior of *atrimandibularis* is the ability to dominate more than one colony simultaneously (CERVO *et al*., 1990c) Females of the other two species, once successfully established on a host nest, remain there, and depend entirely on the host workers to rear the parasite brood Females of *atrimandibularis* engage in extensive extra-colonial activity, during which they usurp nests of the same host species, from which larvae and pupae are taken to the primary (nursery) nest and fed to the brood This behavior occurs with either host species, and CERVO & DANI (1996) wonder whether it evolved originally on *biglumis* nests in response to the short colonial cycle of that species, due to a necessity for collaborating with host to increase fitness, or whether the behavior evolved independently of ecological conditions This predatory behavior may represent a different pathway to obligate parasitism, for example as a form of cuckoo behavior, or it may be a secondary development, for example in response to reduced colony productivity in hosts (CARPENTER *et al.*, 1993)

Cladistic tests

Answers to questions such as those outlined in the preceding section require a cladistic context The justification and a general framework for the use of phylogenetic information in evolutionary biology have been clearly stated by GRANDCOLAS *et al.* (1994) These authors outlined the correspondence between four types of phylogenetic patterns and the evolutionary processes that can be tested with these patterns All are relevant to paper wasp inquilines, to greater or lesser degree, as will be seen

The first correspondence concerns adaptation, which is tested by the phylogenetic pattern of polarity That determining the direction of character change (polarity) is necessary to test whether a feature may be an adaptation is something that has long been understood by cladists (HENNIG, 1966, ELDREDGE & CRACRAFT, 1980) As formulated by CODDINGTON (1988, 1990), adaptation is apomorphic (derived) function, therefore the change from the primitive to the derived condition must be established Direction of change is established by cladistic character optimization, or mapping onto a cladogram I have applied optimization to the question of adaptation in various behavioral features in social wasps before (CARPENTER, 1987, 1988a, WENZEL & CARPENTER, 1994) In the present case, for example, the question whether the predatory behavior of *atrimandibularis* represents a novel pathway to inquilinism, or is secondary, is potentially answered by optimization showing the behavior to be ancestral, or derived.

The second correspondence concerns convergence, which is tested by the phylogenetic pattern of homology and homoplasy Again, the pattern is established by optimization Features

Source : MNHN, Paris

shared by species and shown by optimization on a cladogram to be present in their common ancestor are most parsimoniously inferred to be homologous, that is, to have been inherited from their common ancestor (HENNIG, 1966) Convergence is demonstrated by homoplasy, that is, multiple independent occurrences of a feature on a cladogram A pertinent example is inquiline behavior itself is it homologous (CARPENTER *et al.*, 1993, CHOUDHARY *et al.*, 1994, CARPENTER, 1996a) or has it evolved as many as three times (WEST-EBERHARD, 1996)? If the most parsimonious optimization shows a single origin for this feature, arguments that it has evolved more than once are *ad hoc* (FARRIS, 1983), because there is then no positive evidence for multiple origins Specifically, the claim that a behavior is "labile" and so prone to convergence is contradicted by showing that only a single origin is necessary to explain the evolution of that behavior

The third correspondence is between evolutionary causality and relative phylogenetic appearance Relations among features are often the object of study in comparative biology The relative positions among two or more features shown by optimization on a cladogram may allow test of a causal relation That is, the "time lag" (order of appearance) between origins of features may support or reject a suggested evolutionary progression based on the association of those features I have previously applied this approach to testing a complex evolutionary scenario (CARPENTER, 1989, 1991, 1992) WEST-EBERHARD's (1978) model for the evolution of social behavior in wasps was tested by simultaneously optimizing the different behavioral features onto cladograms for social wasps The relations among these features matched some of the stages in West-Eberhard's model, and did not correspond to others In the case of the evolution of inquilines, a transition from intra- to interspecific usurpation and then obligate parasitism would correspond to part of TAYLOR's (1939) scenario

The fourth correspondence is between adaptive radiation and differential cladogenesis A feature suggested to promote diversification may be optimized onto a cladogram, and the relative diversity of sister-groups compared If the feature in question is ancestral to the larger of two sister-groups, diversification occurred after the origin of the feature, according with the suggestion of adaptive radiation In the case of social parasites, the question is whether inquiline behavior is associated with increased speciation.

All these questions on the evolution of social parasitism may thus be approached by cladistic optimization What is required is a cladogram for the taxa in question, and knowledge of the distribution of behavioral features This raises however the question as to the proper treatment of these features Specifically, should the behavioral data be used during construction of the cladogram, or should they be optimized onto a cladogram constructed independently? That is, would inclusion of these features during cladistic analysis lead to bias or circularity (BROOKS & MCLENNAN, 1991)? KLUGE & WOLF (1993) have argued strongly that features to be interpreted phylogenetically should be used as evidence when inferring phylogeny, and behavioral and ecological features have indeed been treated successfully as characters, that is, as evidence of phylogenetic relationships (reviews in WENZEL, 1992, MILLER & WENZEL, 1995) DELEPORTE (1993) has pointed out that circularity is avoided as long as the cladogram is independent of the evolutionary hypothesis to be tested, not of the characters used That is, the question is not choice of characters, rather it is the rationale that has been used to code the characters Character polarity determined by the cladistic outgroup criterion is independent of hypotheses of direction of change according to an evolutionary model

Source MNHN Paris

But some features are not typically treated as characters. As MICKEVICH & WELLER (1990: 139) put it: "Certain features of organisms are influenced by a large environmental component. The features influenced by environmental factors provide doubtful evidence for phylogenetic relationships." MICKEVICH & WELLER (1990), DELEPORTE (1993) and GRANDCOLAS *et al.* (1994) referred to such features as "attributes" and distinguished them from characters. Attributes are to be interpreted by optimization, rather than used in constructing cladograms. GRANDCOLAS (1993) considered attributes to be features for which a priori homology statements are so problematic that interpretation with reference to a pre-existing phylogeny is preferable. DELEPORTE (1993) and GRANDCOLAS *et al.* (1994) applied DE PINNA's (1991) distinction between primary and secondary homology, suggesting that cladogram construction proceeds only with characters, whose primary (*a priori*) homology may be confidently assessed, while attributes be interpreted, with homology assessed by optimization (secondary homology). However, the distinction between primary and secondary homology should not be maintained dogmatically, because the process of reciprocal illumination ("checking, correcting and rechecking," HENNIG, 1966) is an integral part of cladistics, and the critical test of homology is congruence with other characters. I suggest that logical justification for excluding some features from cladogram construction comes in the distinction between "traits" and characters made by NIXON & WHEELER (1990: 217). These authors defined traits as "attributes that are not universally distributed among comparable individuals within a terminal lineage" and characters as "found in all comparable individuals in a terminal lineage". In this terminology, traits are features that are variably distributed within any grouping (population, species or higher taxon) that is phylogenetically unresolved internally. So for example, features that vary within the genus *Polistes*, such as male clypeal ridges, would be considered traits when the genus *Polistes* is viewed a single terminal lineage. This same feature might diagnose a monophyletic group, and therefore be viewed as a character, if analysis of species within the genus is undertaken. Variable features within populations do not meet the constancy criterion for phylogenetically informative characters. Species are the terminal lineages that are phylogenetically unresolved by definition, because they consist of populations linked by tokogenetic (birth) relationships, to which cladistic analysis is not applicable. Features variable within species are therefore traits by definition. Traits only become phylogenetically informative when they become fixed in comparable semaphoronts (NIXON & WHEELER, 1990; DAVIS & NIXON, 1992), which at the level of species occurs by extinction of plesiomorphic states, that is, at speciation (NIXON & WHEELER, 1992). Features variable within species are precisely those for which *a priori* homology assessment is (by definition) problematic, and such traits should not be used to infer phylogenetic relationships.

To the extent, then, that the behavioral features associated with social parasitism in paper wasps are variable within species, they are best treated as traits. Evolutionary interpretation may be made by optimization on a pre-existing cladogram. Methodologically, the approach taken here will treat all the behavioral features as traits. It will be seen, however, that there are reasons for considering some of these features to be phylogenetically informative characters.

MATERIALS AND METHODS

Taxonomy

Some discussion of taxonomy is first warranted. The nomenclatural history of the European species of *Polistes* is quite tangled (Table 2). Nine species are currently recognized (see CARPENTER 1996b), but four have been described within the past 100 years, and five have gone under more than one name in this century. The type species of the genus *gallicus*, was

Source: MNHN, Paris

misidentified in the revision by KOHL (1898), and thus was followed by other authors until DAY (1979). As a result, the older behavioral literature used the name *gallicus* (or *gallica*) for what is now known as *dominulus*, and *foederata* for what is now known as *gallicus*, including the landmark papers of PARDI (e.g. 1942), which first demonstrated dominance hierarchies in social insects. Numerous subspecies have also been described, but none are more than minor color variants, which do not deserve taxonomic recognition (CARPENTER, 1996b, and see MACLEAN *et al.*, 1978, GUSENLEITNER, 1985, and below).

This nomenclatural instability has had its counterpart in the higher-level taxonomy of these species (CARPENTER, 1996a). The distinctiveness of the social parasites was recognized on the basis of morphology before their behavior was known (ZIMMERMANN, 1930). Discovery of social parasitism led to the generic separation of these three species - under two different names, first, but invalidly, as *Pseudopolistes* (WEYRAUCH, 1937) and then as *Sulcopolistes* (first as a subgenus, BLUTHGEN, 1938; then as a genus, BLUTHGEN, 1943). The latter name was generally used until I (CARPENTER, 1990, 1991, 1996a, VAN DER VECHT & CARPENTER, 1990, CARPENTER *et al.*, 1993) synonymized it with *Polistes*, on the grounds that

TABLE 2 — Taxonomic history of European *Polistes* species.

Author	Currently recognized species				
	biglumis	*dominulus*	*nimphus*	*gallicus*	*associus*
Saussure (1853)	*biglumis*			*gallica*	
Kohl (1898)	*dubia*	*gallica*	*opinabilis*	*foederata*	*associa*
Zimmermann (1930)	*dubia*	*gallica*	*opinabilis*	*foederata*	*chinensis*
Weyrauch (1937)	*dubia*	*gallica*	*opinabilis*	*foederata*	*chinensis* *associa*
Bluthgen (1938)	*kohli*	*gallicus*	*nimpha*	*foederatus*	*associus*
Weyrauch (1938)	*kohli*	*gallica*	*opinabilis*	*foederata*, *omissa*	*associa*
Weyrauch (1939)	*kohli*	*gallica*	*nympha*	*foederata*, *omissa*	*associa*
Blüthgen (1943)	*bimaculatus*	*gallicus*	*nimpha*	*foederatus*, *omissus*	*associus*
Blüthgen (1955)	*biglumis* *bimaculatus*				
Day (1979)		*dominulus*		*gallicus*	
Gusenleitner (1985)				*gallicus*	

Author	Currently recognized species			
	bischoffi	*semenowi*	*sulcifer*	*atrimandibularis*
Saussure (1853)				
Kohl (1898)		*semenowi*		
Zimmermann (1930)		*semenowi*	*sulcifer*	*atrimandibularis*
Weyrauch (1937)	*bischoffi*	*semenowi*	*sulcifer*	*atrimandibularis*
Blüthgen (1938)		*semenowi*	*sulcifer*	
Weyrauch (1938)	*bischoffi*	*semenowi*	*sulcifer*	*atrimandibularis*
Weyrauch (1939)	*bischoffi*	*semenovi*	*sulcifer*	*atrimandibularis*
Bluthgen (1943)	*bischoffi*	*semenowi*	*sulcifer*	*atrimandibularis*

recognition of *Sulcopolistes* rendered *Polistes* paraphyletic. Other generic (*Polistula* WEYRAUCH, 1938) and subgeneric (*Leptopolistes* BLUTHGEN, 1943) names have been proposed for the remaining European species, although they have not always been accepted (RICHARDS, 1973). The misidentification of the type species of *Polistes* means that these other names are synonyms in any event. All of the European species, including the social parasites, are now placed in the subgenus *Polistes*.

Source: MNHN, Paris

sensu stricto (CARPENTER, 1996a, 1996b) *Polistes* is the sole member of the tribe Polistini (CARPENTER, 1993), which is the sister-group of the other Polistinae (CARPENTER, 1991, 1993).

Morphological characters

Morphological characters for the European species of *Polistes*, and two outgroup species are presented in Tables 3-4. Morphological terminology is as in CARPENTER (1996a). All species were examined, including dissection of male specimens for study of genitalia. The literature was surveyed for characters to be examined, in particular keys, both published (*e.g.* GUIGLIA, 1972) and unpublished (STARR & LUCHETTI, 1993). These keys, however, are largely based on color characters which may work well for identification in a limited part of a species' range, but generally show considerable variation over the entire range of widespread species. That was found to be true for the species studied here, particularly when material from *e.g.*, Western Europe was compared to material from Scandinavia or Turkey. Color variation was therefore concluded to be microenvironmentally induced (see MACLEAN *et al.* 1978), and was not included. Some of the morphological characters discussed in the literature were also found to be more continuously variable than implied by their inclusion in keys (for example, the characters used to separate *foederatus* and *omissus*, both now considered synonyms). The character states listed in Table 4 are those that could be consistently distinguished. Additivity of multistate characters was determined from observed nested similarity.

Tables 3-4 also include characters establishing monophyly of the ingroup, namely those establishing monophyly of the subgenus *Polistes sensu stricto* relative to the outgroups, both of which are species in the New World subgenus.

TABLE 3. Morphological characters for European *Polistes* species, and two outgroups. Multistate characters are treated as additive except for character 18. See Table 4 for Matrix.

1. MALE ANTENNAE: tapered (0), coiled (1). This is a synapomorphy for the subgenus *Polistes sensu stricto* in CARPENTER (1996a).
2. MALE ANTENNAL APEX: rounded (0), pointed (1). A number of other distinctions in shape of the terminal article of the male antenna have been made since the time of KOHL (1898), but do not appear to be tenable.
3. SCAPE: cylindrical (0), dorsobasally flattened (1).
4. MALE INTERANTENNAL RIDGE: raised (0), grooved (1).
5. CLYPEAL APEX: convex (0), angular (1), broadly depressed (2). Depression of the clypeal apex diagnoses the inquilines, and is variable among the species, being more extreme in *sulcifer*.
6. MALE CLYPEAL RIM: smooth (0), angulate rim (1). Interpretation of this character is complicated. It appears to be derived within the genus *Polistes* as a whole, in the European species, but then appears to have been lost in the group of species related to *gallicus* (see Fig. 3).
7. MALE CLYPEAL RIDGES: absent (0), present (1), extending to clypeal apex (2). The angulate, black rimmed margin of the clypeus seems to be an exaggeration of slight swellings commonly seen in males of the subgenus *Polistes sensu stricto*, and fully developed ridges are seen in some of the non-European species.
8. MALE CLYPEAL PUNCTATION: weak (0), macropunctures (1).
9. MALE CLYPEAL PROPORTIONS: width > length to width = length (0), length > width (1). As the coding indicates, this character shows some continuous variation, which does not seem to lend itself to further partitioning.
10. MALAR SPACE: gena tapering (0), gena quadrate (1). The enlarged malar space is diagnostic of the inquilines.
11. MANDIBLE: smooth (0), shallow groove (1), ridges pronounced (2), deeply grooved (3). Excavation of the external surface of the mandible is diagnostic of the inquilines, and is variable among the species, showing a morphocline in development. The groove is shallow in *atrimandibularis*, and *semenowi* and *sulcifer* are more similar to one another in having more perpendicular ridges, which are greatly exaggerated in *sulcifer*.
12. MALE OCCIPUT: convex (0), straight (1). Temples convergent backwards is supposed to diagnose the species formerly placed in *Leptopolistes* (viz. *associus*, *bischoffi* and *gallicus non auct.*) - but is found in non-European species of the subgenus *Polistes sensu stricto*.
13. MESEPISTERNAL PUNCTATION: fine (0), clathrate (1). This is a synapomorphy for the subgenus *Polistes sensu stricto* in CARPENTER (1996a).
14. EPICNEMIAL CARINA: absent (0), present (1). This is a synapomorphy for the subgenus *Polistes sensu stricto* in CARPENTER (1996a). However, it is reduced to traces in *bischoffi*.
15. SCUTAL HAIRS: short (0), elongate, much longer than an ocellus diameter (1). This character appears to vary, but some of this variation is evidently due to specimen wear.
16. PARAMERAL SPINE: straight (0), lobed (1).
17. AEDEAGAL TEETH: fine (0), robust (1).
18. AEDEAGUS MEDIAL LOBES: small, sharp (0), large (1); square (2); pointed (3).

Source: MNHN, Paris

TABLE 4 Morphological characters for European *Polistes* species, and two outgroups Multistate characters are treated as additive except for character 18

Taxon	111111111 123456789012345678
exclamans	000000000000000111
dorsalis	000000000000000000
biglumis	101101000000111111
dominulus	111101010000110111
nimphus	101101100000110111
gallicus	101100101001110113
sulcifer	101121000130110112
atrimandibularis	101111010110110111
semenowi	101111000120110111
associus	101100201001110111
bischoffi	101100101001101111

Aphanilopterus sister-group of *Polistes sensu stricto* (CARPENTER, 1996a) The two outgroup species included, *dorsalis* and *exclamans*, were selected because they were used in both the molecular analyses (CARPENTER *et al* , 1993, CHOUDHARY *et al* , 1994)

Cladistic Procedures

Cladistic analysis (HENNIG, 1966) was implemented with the computer program Nona (GOLOBOFF, 1996a) As pointed out elsewhere (CODDINGTON & SCHARFF 1994 CARPENTER, 1996a), Nona implements a more stringent requirement for cladogram support than other available programs cladograms are only reported if every branch is supported by all possible optimizations of at least one character Programs such as Hennig86 (FARRIS, 1988) report cladograms with branches supported only by one of several possible optimizations for at least one character Such optimizations can lead to the program reporting cladograms that are not in fact supported by the data with branches supported by optimizations that cannot simultaneously coexist with other branches (examples are given in LORENZEN & SIEG, 1991, CODDINGTON & SCHARFF, 1994, WILKINSON 1995 CARPENTER, 1996), even without missing values which can also produce this misleading result (PLATNICK *et al* , 1991) CODDINGTON & SCHARFF (1994) argued that branches supported only under some optimizations are desirable, but such "semistrict" support is at best ambiguous, if not misleading (NIXON & CARPENTER, 1996b)

The Nona program's implementation of "strict" support (NIXON & CARPENTER, 1996b) is incomplete, because the program does not optimize on multifurcations Hence, it does not necessarily collapse all ambiguously supported branches Several methods of collapsing such branches may be implemented with current programs (NIXON & CARPENTER, 1996b); with Nona, use of the "ksv" command to save cladograms in collapsed form, reading them back into Nona, and issuing the "best" command will filter out semistrict support That method is employed here Output from Hennig86 is also reported, for comparison For both programs, exact calculations were made, using either the "mswap" command of Nona or the "ie" command of Hennig86

Character weighting was employed as a check of the reliability of the results both successive weighting as implemented in Hennig86, and implied weighting as implemented in the program Piwe (GOLOBOFF, 1996b) *A posteriori*, recursive character weighting checks the self-consistency of results a cladogram based on reliable characters should imply weights that imply the same cladogram (see CARPENTER, 1988b, 1994, CARPENTER *et al.*, 1993).

In the simultaneous analyses of the morphological characters, allozymes and mtDNA sequences, the allozyme data were taken from tables 2-4 of CARPENTER *et al* (1993), while the mtDNA data of CHOUDHARY *et al* (1994) were provided by J F STRASSMANN CHOUDHARY *et al* (1994) reported 79 polymorphic sites, of which 55 were potentially informative out of 386 sites sequenced Their aligned sequences were converted to files for analysis by Nona and Hennig86 by the program MacAlign (WHEELER & GLADSTEIN, 1995) due to differences in interpretation of ambiguity codes, this resulted in 93 polymorphic sites (of which 43 were informative) This difference was irrelevant to the results, as analysis produced the same

Source MNHN, Paris

two cladograms reported by CHOUDHARY *et al* (1994) The program Dada (NIXON, 1995a) was used to calculate the incongruence length difference (FARRIS *et al*, 1994) among the character sets, and to determine whether these values were large, by performing the significance test of FARRIS *et al* (1994) For the tests 100 iterations were made using Hennig86 for cladogram calculations on the random matrix partitions

Optimizations of the behavioral traits listed in the following section were calculated with the program Clados (NIXON, 1995b) which was also used to print results The "allstates" command was used to check all possible optimizations at each node of the cladograms

Behavioral Traits

Behavioral traits pertinent to social parasitism are listed in Table 5 These traits or attributes were compared among social parasites and their hosts in the discussions of the evolution of social parasitism by CERVO *et al* (1990b) and CERVO & DANI (1996) As the scorings in Table 6 show, all of the variables are variable within species, aside from colony cycle length (which is known to vary intraspecifically, YAMANE 1996) The questions that I will attempt to answer by optimization are stated for each trait below

TABLE 5 Behavioral traits for European *Polistes* species, related to social parasitism Multistate trait 4 is treated as nonadditive See Table 6 for Matrix

1 FOUNDATION: haplometrosis (0); pleometrosis (1)
2 CYCLE LENGTH: long (0), short (1)
3. USURPATION: intraspecific (0), interspecific (1)
4 INQUILINISM: absent (0); parasitism (1), predation (2)
5 TIMING: pre-emergence (0); post-emergence (1)
6 USURPATION TACTICS: aggressive (0); passive (1)

TABLE 6 — Matrix of behavioral traits for European *Polistes* species, related to social parasitism Multistate trait 4 is treated as nonadditive A question mark denotes an unknown state, while a dash denotes an inapplicable trait A asterisk denotes a polymorphism showing all applicable states, a dollar sign denotes a subset polymorphism (1,2 for *atrimandibularis*) See Table 5 for list of traits

Taxon	123456
exclamans	*00000
dorsalis	*0?0--
biglumis	010000
dominulus	10*000
nimphus	10*000
gallicus	000000
sulcifer	--11*0
atrimandibularis	--1$01
semenowi	--11**
associus	???0--
bischoffi	???0--

Colony foundation Whether exclusively haplometrotic or also pleometrotic The relation of this to origin of obligate parasitism is of interest (BUSCHINGER, 1986, 1990, CARPENTER *et al*, 1993). As noted in CERVO & DANI (1996), while intraspecific usurpation occurs both in colonies with nest foundation by a single female (haplometrosis) and with multiple foundresses (pleometrosis), usurpation is less successful in pleometrotic colonies Scorings for the outgroups, which are polymorphic, are drawn from the following references for *dorsalis*, SPIETH (1948, cited as *fuscatus hunteri*), and STRASSMANN (pers com), for *exclamans*, STRASSMANN (1981) Note that even *biglumis* exhibits pleometrosis occasionally (LORENZ, &

Source MNHN, Paris

TURILLAZZI 1986), although that is not scored here The question is did inquiline behavior originate in haplometrotic or pleometrotic ancestors?

Colony cycle length Whether long or short Factors influencing the extensive variation in this trait were reviewed by Yamane (1996) Is a short colony cycle derived, the minimal requirement for interpretation as an adaptation?

Usurpation Whether it is intraspecific or interspecific See CERVO & DANI (1996 table 5 1), for a list of *Polistes* species in which intraspecific usurpation has been reported They also reported (CERVO & DANI, 1996 104) observing two cases of interspecific usurpation in *dominulus*, and CERVO (pers com) has recently observed the phenomenon in *nimphus* These two species are therefore scored as polymorphic Is interspecific usurpation derived from intraspecific usurpation?

Inquilinism Whether it is absent or present, and whether it is accompanied by predation Unlike the other behavioral features discussed here inquiline behavior as such does not show intraspecific variation It is constant and so may be treated as a character WEST-EBERHARD (1996) nevertheless argued that it may have arisen in all three socially parasitic species independently, whether they form a monophyletic group or not That is *ad hoc*, as pointed out above But whether or not the predatory behavior of *atrimandibularis* is primitive or derived is a question of interest, and that behavior is found within one species, which also exhibits parasitic behavior like the other two inquilines The feature is therefore treated as a trait, polymorphic for both parasitism and predation in *atrimandibularis* What is the association between origin of inquilinism and transitions in usurpation?

Timing of invasion Whether pre-emergence or post-emergence Is the early timing of invasion by *atrimandibularis* derived, the minimal requirement for interpretation as an adaptation?

Usurpation tactics Whether aggressive or initially passive The scoring includes polymorphism within *semenowi* which in one case has been observed to show passive tactics (DEMOLIN & MARTIN, 1980) Are the initially submissive tactics of *atrimandibularis* derived, the minimal requirement for interpretation as an adaptation?

RESULTS

Morphology

Analysis of the data in Tables 3-4 with Nona (using "ms 8") resulted in two cladograms, with Hennig86, 12 cladograms were reported The length is 28, the consistency index is 0 85 and retention index is 0 84 (see FARRIS, 1989, for definition of the indices) The consensus tree for either set of cladograms is shown in Fig 3, this tree shows the groups in common to all of the cladograms (such consensus trees are also referred to as "Nelson" or "strict") Implied weighting, using the default weighting function of Piwe, resulted in the same two cladograms as reported by Nona Successive weighting on the 12 cladograms reported by Hennig86 resulted in a report of six cladograms; their consensus is also Fig 3.

The morphological characters do not completely resolve the interrelationships among the European species, as is the case with the two published molecular data sets However, the morphological data do resolve interrelationships among the three socially parasitic species, unlike the molecular data sets The morphological data set also allowed inclusion of all the European species, again unlike the molecular data sets, which could not include *associus* and *bischoffi* This demonstrates that *gallicus* and *biglumis* are in fact not closely related These two species were resolved as sister-groups by the mtDNA data and the independent allele coding of the allozyme data The morphological characters show that *associus* and *bischoffi* are more closely related to *gallicus*

Simultaneous analyses

None of the three available data sets completely resolves the interrelationships of the European species, but the combination of the three matrices does so Simultaneous analysis of combined data, which has been called "total evidence" (KLUGE, 1989) is currently controversial in cladistics, with some authors arguing against combining data sets (*e.g.* BULL *et al.*, 1993, DE QUEIROZ, 1993, MIYAMOTO & FITCH, 1995) All such arguments are without force, as discussed in NIXON & CARPENTER (1996a) And a decisive argument justifies combining data sets

Source MNHN, Paris

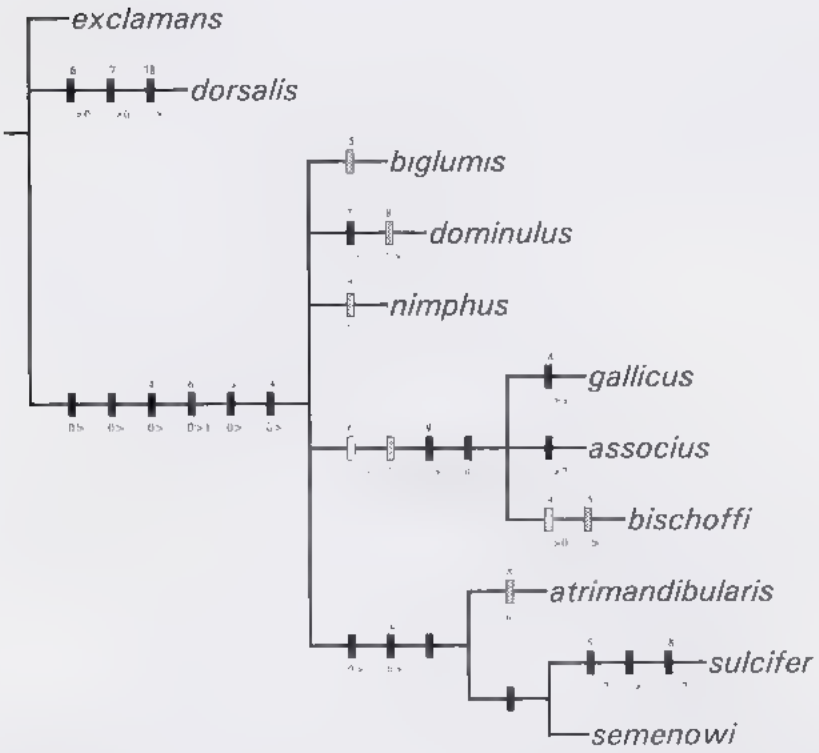


FIG 3 — Consensus tree for cladograms reported after exact analysis of the character matrix in Table 4, by either Nona or Hennig86. This is also the consensus for the six cladograms resulting from successive weighting by Hennig86. Characters have been optimized by the default "slow" transformation as implemented in Clados. Character numbers are above the hashmarks, state changes are shown below, with the respective primitive and derived conditions separated by a ">". Filled hashmarks denote unique origins, grayscaled hashmarks indicate convergent changes, and open hashmarks are reversals.

and determining the most parsimonious solution for all the data seeks the cladogram that is best supported and maximally explanatory for all the data. This approach maximizes information content and corroboration of the resulting hypothesis.

The morphological data from Tables 3-4 were combined with the allozyme data from CARPENTER *et al.* (1993) and the mtDNA data from CHOUDHARY *et al.* (1994). Three separate analyses of the combined morphological and molecular data were undertaken, corresponding to the three different coding schemes for the allozymes presented by CARPENTER *et al.* (1993, tables 2-4). The same cladogram resulted from analysis with Nona regardless of the allozyme coding scheme employed, it is shown in Fig. 4. This cladogram also resulted from implied weighting with Piwe. Hennig86 reported three cladograms regardless of which allozyme coding scheme was employed, these cladograms were stable to successive weighting. The consensus of the three cladograms is also Fig. 4. The three cladograms differed only in resolving relationships among *gallicus*, *associus* and *bischoffi*, based entirely on the possible optimizations for the numerous missing values found in the latter two species, hence three cladograms are not actually supported by the data.

Source: MNHN, Paris

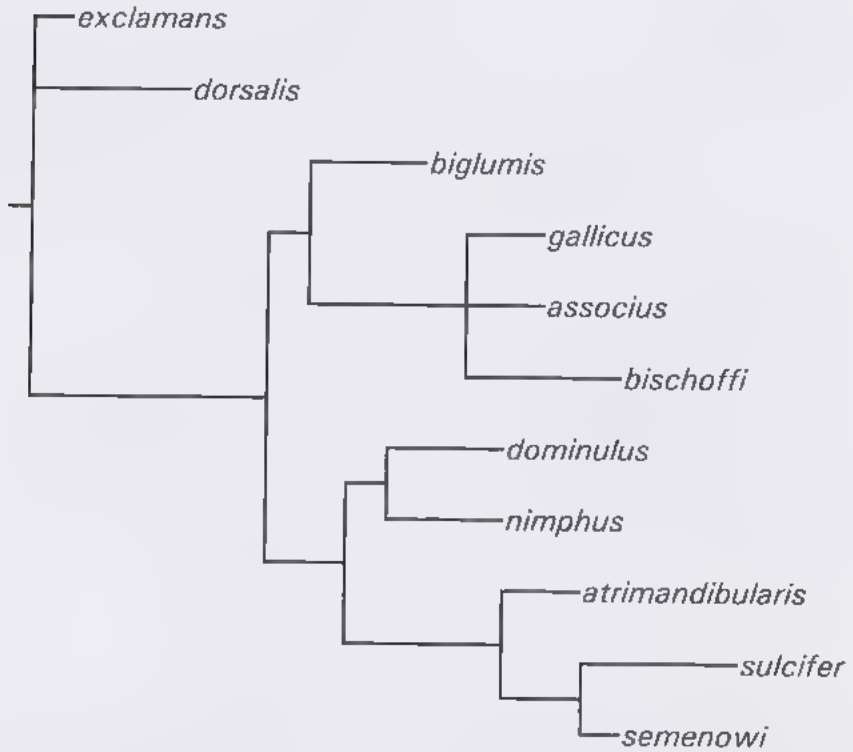


FIG. 4. — Cladogram resulting from simultaneous analysis of morphological and molecular data

For the independent alleles coding from CARPENTER *et al.* (1993: table 2), analysis of 220 characters resulted in Fig. 4 with a length of 271, consistency index 0.83 and retention index 0.72. For the multistate locus coding from CARPENTER *et al.* (1993: table 3), analysis of 138 characters resulted in Fig. 4 with a length of 222, consistency index 0.90 and retention index 0.81. For the minimum turnover coding from CARPENTER *et al.* (1993: table 4), analysis of 148 characters resulted in Fig. 4 with a length of 229, consistency index 0.87 and retention index 0.77.

The results from incongruence length difference testing are as follows. For the independent alleles coding, the incongruence length difference is 3, and the percentage of 100 iterations reported by Dada as equaling or exceeding that value is 37. For the multistate locus coding, the incongruence length difference is 1, and the percentage of 100 iterations reported by Dada as equaling or exceeding that value is 83. For the minimum turnover coding, the incongruence length difference is 2, and the percentage of 100 iterations reported by Dada as equaling or exceeding that value is 75. The null hypothesis of congruence accordingly fails rejection by quite large margins for any combination of morphology, mtDNA and allozyme coding.

Optimizations

Optimization of the behavioral traits is shown in Figs. 5-14. Changes are shown by hashmarks on the branches of the cladograms. All of the traits are mapped in Figs. 5-6, which show two different, equally parsimonious optimizations. In Fig. 5, the traits are mapped

Source: MNHN, Paris

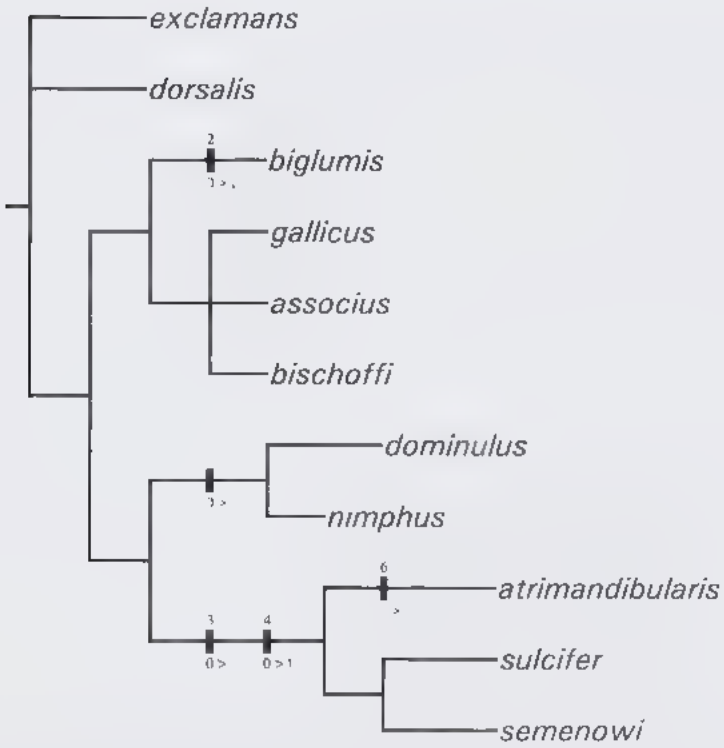


FIG 5 — Cladogram showing optimization of behavioral traits related to social parasitism The default, slow optimization, is shown Plotting conventions as in Fig 3

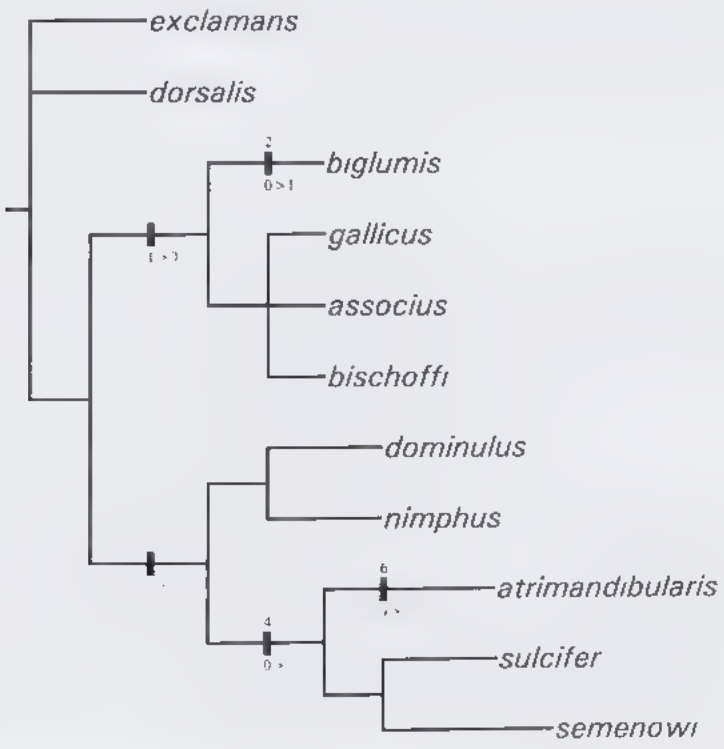


FIG 6 Cladogram showing alternative, equally parsimonious 'fast' optimization Plotting conventions as in Fig 3

Source MNHN, Paris

according to the default optimization in Clados, "slow" transformation, which places changes toward the tips of the cladogram This procedure is similar to delayed transformation (or "deltran", *e.g.* SWOFFORD & MADDISON, 1987) In Fig 6, the traits are mapped according to the alternative, "fast" transformation of Clados, which places changes toward the root of the cladogram This procedure is similar to accelerated transformation (or "acctran", SWOFFORD & MADDISON, 1987).

Optimization of the first trait, whether colony foundation is haplometrotic or pleometrotic, is ambiguous, and its placement is one difference among the two figures The reason for this ambiguity is shown in Figs 7-8, which show two possible placements for this trait Because the trait is polymorphic in both of the outgroups, either state may be assigned to the common ancestor of the ingroup. Moreover, because the trait is inapplicable to the social parasites (which do not have colony foundation behavior), the optimization procedures, which treat inapplicable values as missing data, consider either state possible for them, and so the step in this trait could even be assigned to the common ancestor of the social parasites and their sister-group, *dominulus* + *nimphus* The "squeeze missing data" optimization of Clados, which places changes so that the fewest number of taxa with missing values are included above the step, may be used to filter out such possibilities

One other trait shows a similar difference in placement according to optimization usurpation, whether it is intraspecific or interspecific Figs 10-11 show the alternative slow and fast optimizations Because the trait is polymorphic in *dominulus* and *nimphus*, either state can be assigned to their common ancestor, and thus to the common ancestor of the social parasites and their sister-group

Mapping of the other four traits is the same regardless of the optimization procedure Optimizations of the individual traits are shown in Figs 9 and 12-14 Fig 13 shows no changes in timing of invasion This trait is polymorphic in *sulcifer* and *semenowi*, therefore the only changes are parsimoniously placed within terminal lineages Such changes are not mapped by Clados

DISCUSSION

The social parasites in *Polistes* form a monophyletic group They are thus not most closely related to any of their hosts, and EMERY's Rule is rejected Both the morphological characters analyzed in this paper and mtDNA data (CHOUDHARY *et al.*, 1994) establish monophyly unequivocally, and combination of these data with allozyme data (CARPENTER *et al* , 1993) lead to the same result EMERY's Rule having been dispensed with, arguments that sympatric speciation was involved in the origin of the inquilines from their hosts are rendered moot in paper wasps

This conclusion raises the issue of whether the inquiline behavior arose once, or several times The parsimonious optimization of this behavior (Fig 12) indicates a single origin WEST-EBERHARD (1996) argued for the possibility of multiple origins despite monophyly of the inquilines There is no evidence for this, but WEST-EBERHARD (1996 315) stated: "The law of parsimony requires attributing the common features of a monophyletic group to their common ancestry only in the absence of biological evidence to the contrary The evolutionary lability of facultative traits, and their propensity to reversals within a lineage as well as to rapid and parallel

Source MNHN, Paris

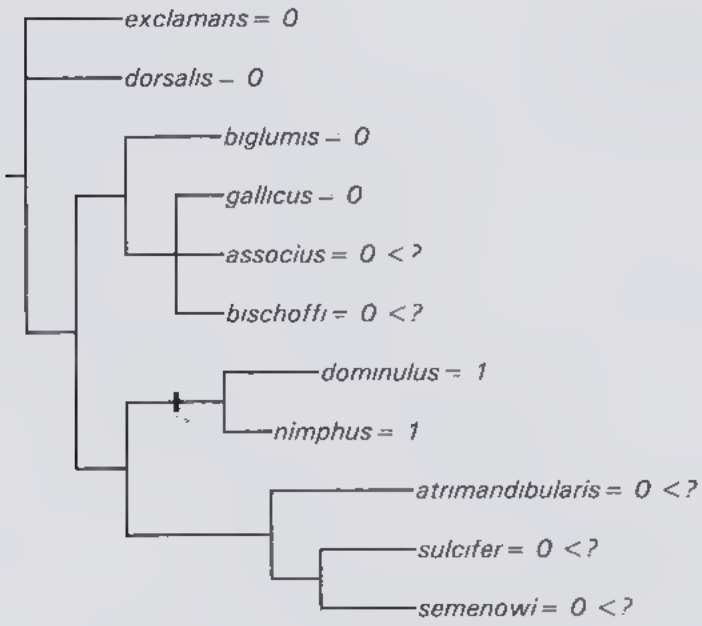


FIG 7 Cladogram showing slow optimization of colony foundation. Plotting conventions as in Fig 3. States of each taxon are shown next to the taxon name, separated by an "=" sign. For taxa with unknown or inapplicable values, the state possible according to the optimization procedure is shown, with "< ?" written next to the state to indicate that the value is actually missing.

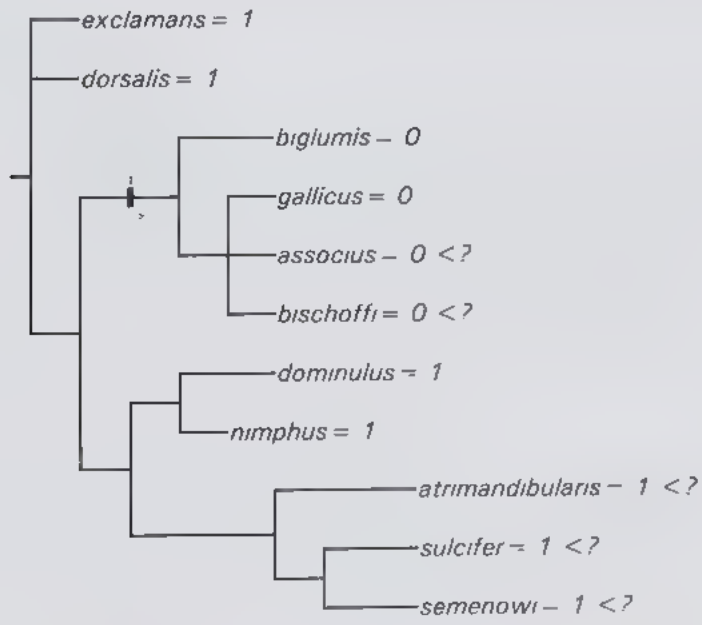


FIG 8 — Cladogram showing fast optimization of colony foundation.

Source: MNHN, Paris

fixation in related species give reason at least to consider the possibility of multiple fixations of obligate parasitism in *Polistes*. Evolutionary lability of conditionally expressed traits means that it may sometimes be impossible confidently to track character "fixation" points using cladistics methods, because the character can come and go (change polarity) and rapidly become fixed rather than optional *between* the branching points of a cladogram (see also FRUMHOFF & REEVE 1994)".

Several points may be made to counter this argument. WEST-EBERHARD considered inquiline behavior to be evolutionarily labile. It is not the social parasites are fixed for this feature, it does not vary in its expression within the species, nor is it found in other species. This observation also indicates the weakness in the argument of FRUMHOFF & REEVE (1994) cited by WEST-EBERHARD. FRUMHOFF & REEVE concocted a probabilistic model to show that inference of ancestral character states by optimization is highly error-prone. Their model may be rejected immediately as unrealistic (see general argument in FARRIS, 1983), but even if their approach is accepted, FRUMHOFF & REEVE failed to realize that observation of fixation for a character in a clade provides information on the evolutionary rate of change (SCHULTZ *et al.*, 1996), and specifically against the conclusion that a character has undergone a large amount of change. FRUMHOFF AND REEVE's argument is thus generally irrelevant to accuracy of ancestral conditions as inferred by optimization (SCHULTZ *et al.*, 1996).

Because inquilinism is not evolutionarily labile, there is no reason to entertain the *ad hoc* notion that it has arisen in parallel within a monophyletic group. Social parasitism indeed evidently arose from intraspecific usurpation (Figs. 5-6, and see below), which may be considered a "phenotypic alternative" to independent nesting (WEST-EBERHARD, 1996). Even so, inquiline behavior became fixed at some point, and there is no necessity to postulate that event occurring more than once. Inquilinism is in this view really a character, providing evidence that the species showing the feature form a monophyletic group. This conclusion also applies to the morphological characters of the social parasites. The depressed clypeal apex, quadrate malar space and grooved mandible shared by the parasites are interpreted as synapomorphies in Figs. 3-4. These characters might be dismissed as evidence of phylogenetic relationship because they are considered to be adaptations to parasitism, involved in domination of the host during usurpation. WEST-EBERHARD (1996: 316) referred to these characters as "morphological accoutrements of parasitism" and suggests that in the inquilines these characters "should not, of course, be taken as evidence that obligatory parasitism occurred prior to their speciation since these characteristics can be convergent (or parallel) developments".

Whether these features arose as adaptations or not, they became fixed, constant in distribution, restricted to the inquilines and occurring in no other species. These characters show no geographic variation whatsoever, contrary to the prediction of WEST-EBERHARD (1996: 313). There is no necessity for supposing this fixation to have occurred more than once, because the inquilines are a monophyletic group, as established by both morphology and molecular data (Fig. 4). Moreover, two of the characters show transformation series. The depression of the clypeal apex evidently evolved from slightly to broadly flattened (character 5 on Fig. 3), and the mandibular groove evidently changed from shallow to having pronounced ridges, to being deep

Source: MNHN, Paris

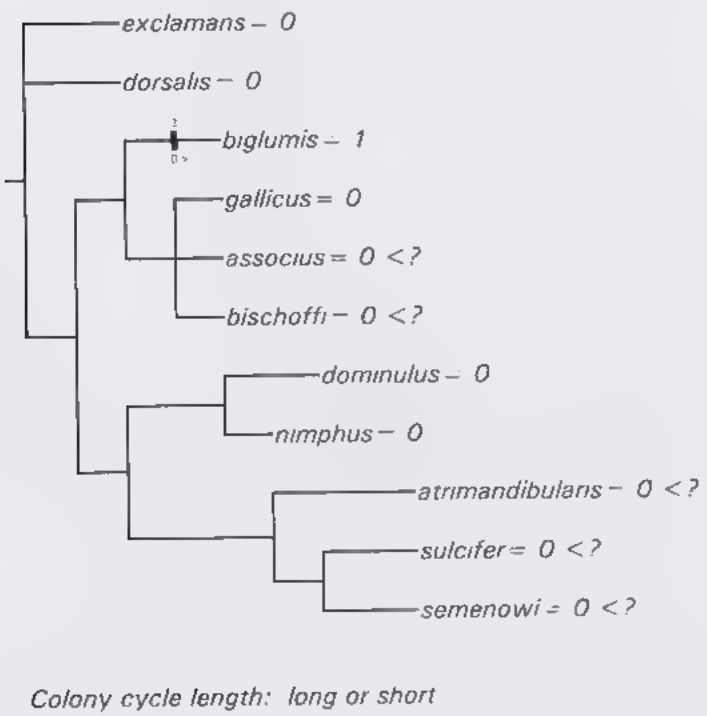


FIG 9. — Cladogram showing optimization of colony cycle length

(character 11 on Fig 3) These transformations occurred as the inquilines speciated, and indicate that the common ancestor had the morphological accoutrements of parasitism Therefore, presumably, it also had inquiline behavior.

The optimizations in Figs 5-6 accord with the central idea underlying TAYLOR's (1939) scenario, but do not support the scenario as a whole Interspecific usurpation evidently evolved from intraspecific usurpation (Figs 10-11) Intraspecific usurpation in *Polistes* is facultative and temporary (stage 1 in TAYLOR's scenario), and preceded the obligate and permanent usurpation of the inquilines (stage 4 in the scenario) But is unclear whether facultative and temporary interspecific usurpation preceded inquiline behavior Temporary interspecific usurpation has been reported as a facultative behavior extremely rarely, but does occur in the sister-group to the social parasites (*dominulus* + *nimphus*) The optimization of this behavior is therefore ambiguous inquiline behavior could have evolved directly from intraspecific usurpation (Fig 10) or via an intervening stage of temporary interspecific usurpation (Fig 11), which corresponds to stage 2 in the scenario.

In either case, the other stage in TAYLOR's scenario, which is obligate and temporary interspecific usurpation, does not correspond to the behavioral changes shown in Figs 5-6 Interspecific usurpation is either facultative and temporary, or obligate and permanent Indeed, temporary interspecific usurpation has not been observed as obligate behavior WEST-EBERHARD (1996 313) suggested "In *Polistes* temporary obligate social parasitism, in which the usurper produces some worker offspring of her own, is expected to be rare or absent if [ ] obligate (interspecific) parasitism originates in extreme climates with short nesting seasons, and invasion

Source MNHN, Paris

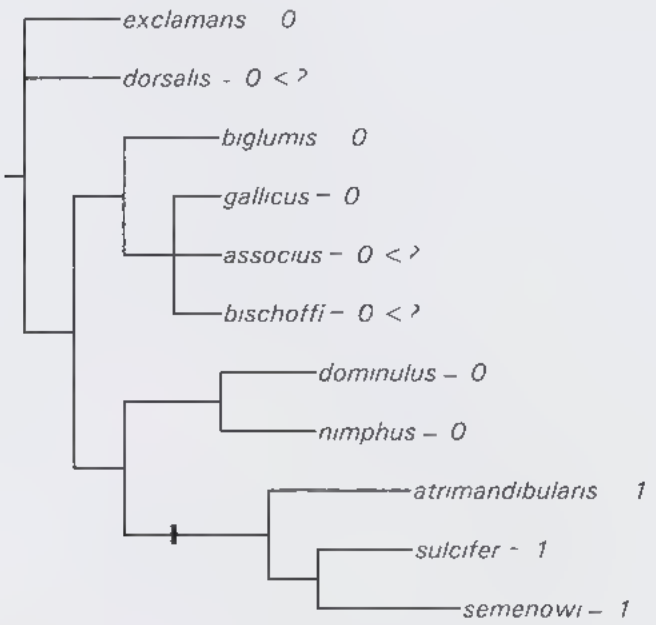


FIG. 10 — Cladogram showing slow optimization of usurpation

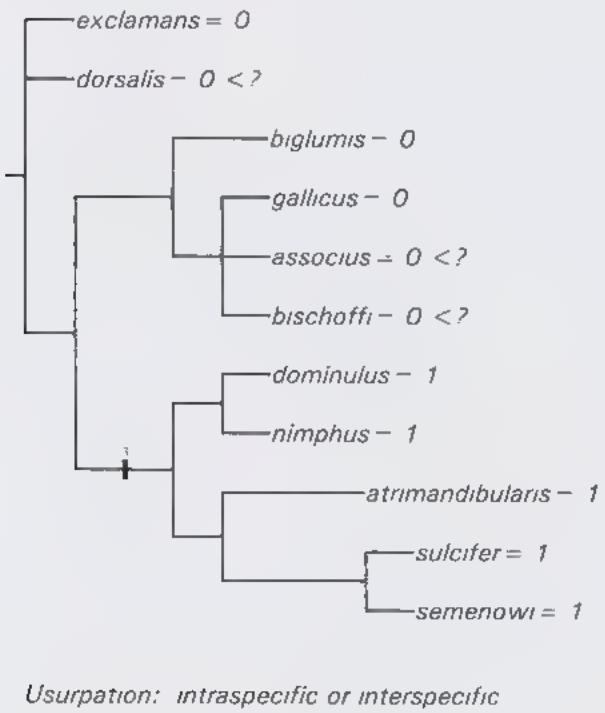


FIG 11. — Cladogram showing fast optimization of usurpation

Source MNHN, Paris

occurs at about the time of first worker emergence [ ] At that time temperate-zone *Polistes* females are already beginning to lay reproductive-producing eggs [ ] Usurpers in such a species would soon produce reproductive offspring, not workers, and "temporary" parasitism may seldom occur" WEST-EBERHARD (1996 314) concluded "Temporary parasitism is not a necessary step in the transition from facultative to obligatory parasitism Nor is facultative interspecific parasitism expected to be common [ ] it may occur only when usurpers are running out of conspecific host colonies in a population where usurpation is the most frequently productive option – a transient situation expected to change rapidly toward obligate parasitism in the presence of suitable hosts"

The third stage of TAYLOR's scenario is thus unnecessary (as is also in fact the second)

What answers to the other questions posed about behavioral traits may be provided by the optimizations? Taking the traits in turn, whether the inquiline behavior originated in a haplometrotic or pleometrotic ancestor cannot be answered the optimization is ambiguous (Figs 7-8) Given the intraspecific variation in this trait, the question may indeed be unanswerable

A short colony cycle is evidently derived in *biglumis* (Fig 9), and so is a candidate for being an adaptation But whether this has anything to do with the evolution of inquiline behavior is not at all clear As plausible as may be the notion that inquilines arose from an ancestor with a shortened cycle (LORENZI & TURILLAZZI, 1986, CERVO & DANI, 1996, WEST-EBERHARD, 1996), it is not evident whether that was indeed the condition of the primitive host If host species were treated as a trait and optimized on the cladogram, *dominulus* may be inferred to have been the ancestral host for *semenowi* and *sulcifer*, but the ancestral host for inquilines as a

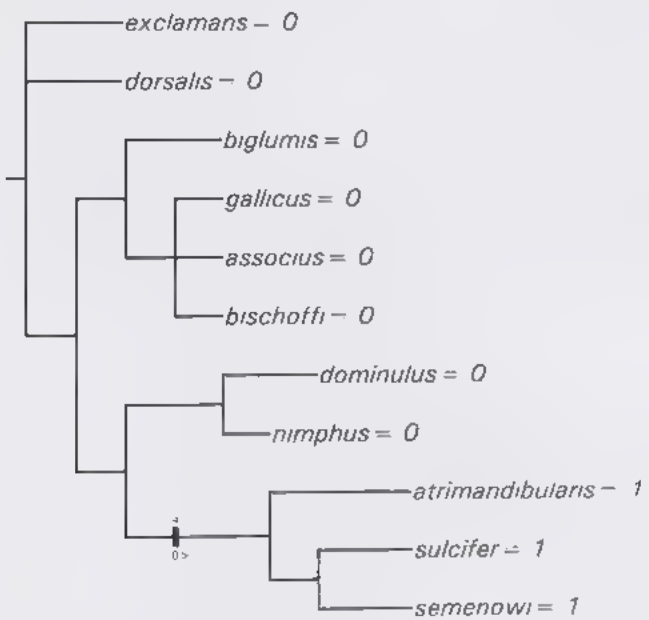


FIG 12. — Cladogram showing optimization of inquilinism

Source MNHN, Paris

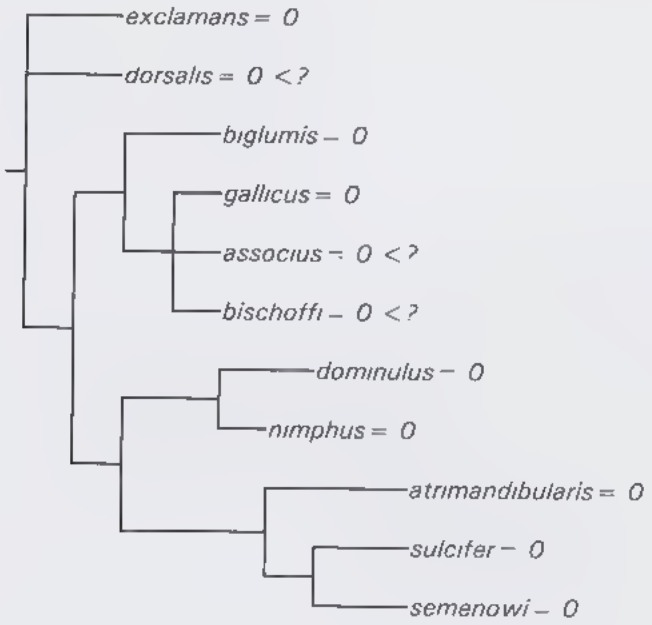


FIG 13. — Cladogram showing invariance of timing of invasion

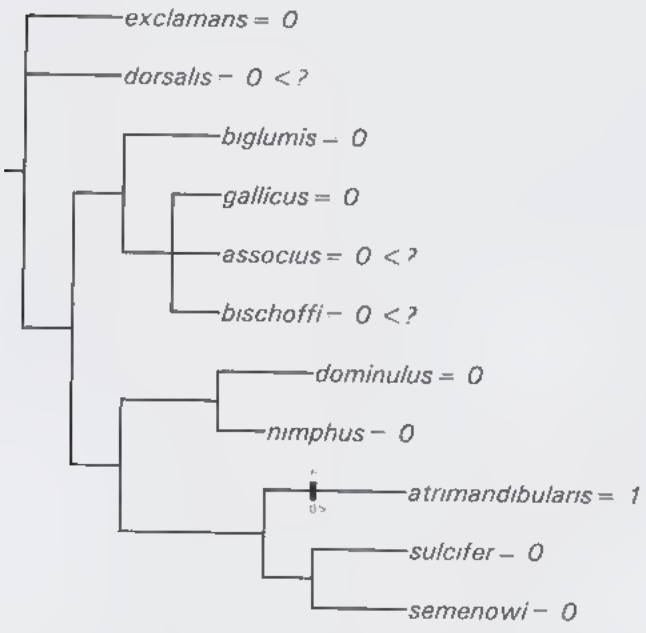


FIG 14 — Cladogram showing optimization of usurpation tactics.

Source MNHN, Paris

whole could be *dominulus*, *gallicus* or *biglumis* At least the possibility that the ancestral inquiline had a host like *biglumis* is not excluded

The predatory behavior shown by *atrimandibularis* is evidently derived from parasitism (Fig 12), occurring only within that species It thus does not represent a different pathway to obligate parasitism, the behavior is a secondary development This does not answer the question as to whether the behavior originated as an adaptation to the shortened colony cycle of its host species *biglumis* (CERVO & DANI, 1996), but leaves the possibility open However, if predatory behavior is a specific adaptation to the host *biglumis*, that would not seem to accord very well with the notion that *biglumis* represents the primitive host for the inquilines (CERVO & DANI, 1996), or that parasitism arose in such a species (WEST-EBERHARD, 1996) The adaptation is not primitive in inquilines, it is derived within *atrimandibularis*.

The post-emergence timing of invasion shown by *semenowi* and *sulcifer* is evidently derived from pre-emergence invasion, arising separately within each of those species (Fig 13) The pre-emergence timing of invasion shown by *atrimandibularis* is thus still a candidate for being an adaptation to the shortened cycle of its host *biglumis* (CERVO *et al.*, 1990c) – but it is a marginal candidate, being viable only if *biglumis* was the original host of the inquilines

Finally, regarding the question of usurpation tactics, the passive tactics shown by *atrimandibularis* are evidently derived (Fig 14), from primitively aggressive tactics Initially passive behavior is thus specialized (CERVO & DANI, 1996) It seems a better candidate for an advanced form of parasitism than a prerequisite for parasitism – but, again, the conclusion of derived behavior does not fit very well with the notion that *biglumis* or something like it as the original host of the inquiline lineage

In summary, the cladistic approach to the evolution of social parasitism in paper wasps provides decisive tests of the generalization known as EMERY's Rule, and TAYLOR's scenario for the origin of inquiline behavior It provides answers to some of the questions posed on the evolution of various features of parasitic behavior, and is ambiguous on others In terms of the framework for the use of phylogenetic information in evolutionary study developed by GRANDCOLAS *et al.* (1994), the present application illustrates all four of the phylogenetic patterns corresponding to tests of evolutionary processes Adaptation is a possible explanation for features polarized as derived, such as the predatory behavior of *atrimandibularis* - although comparative functional studies remain to be done Convergence is an unnecessary explanation for features optimized as derived once, like the origin of inquiline behavior A causal connection is corroborated by association between particular traits, for example interspecific usurpation that is both permanent and obligate, showing no time lag in relative appearance Various reasons may be advanced for such a connection (see WEST-EBERHARD, 1996), although tests may be difficult to formulate And adaptive radiation, shown by differential cladogenesis, is a possible consequence of the origin of inquiline behavior The inquiline clade, with three species, is not large, but is more diverse than its sister clade, *dominulus* + *nimphus* Yet whether that difference is construed as impressive or not, the taxonomic context is incomplete The European species of *Polistes* are not the only members of the subgenus *Polistes sensu stricto* (*sensu* CARPENTER, 1996a, 1996b) It is possible therefore that some of the African and Asian species of the subgenus are closely related to the *dominulus* + *nimphus* clade, similar to the situation of close relationship of *associus* and *bischoffi* to *gallicus*, which remained uninvestigated by the previous cladistic analyses based on molecular data Further study of the phylogenetic relationships among

Source MNHN, Paris

the species of the subgenus is required to address this question And this should be accompanied by more extensive study of the behavioral ecology of these species As GRANDCOLAS *et al.* (1994 671) concluded "Finally, we submit the plea that more phylogenies are needed for comparative studies Taxonomies should not be used for want of something better Fruitful collaborative or integrated works should be carried out to achieve comparative studies"

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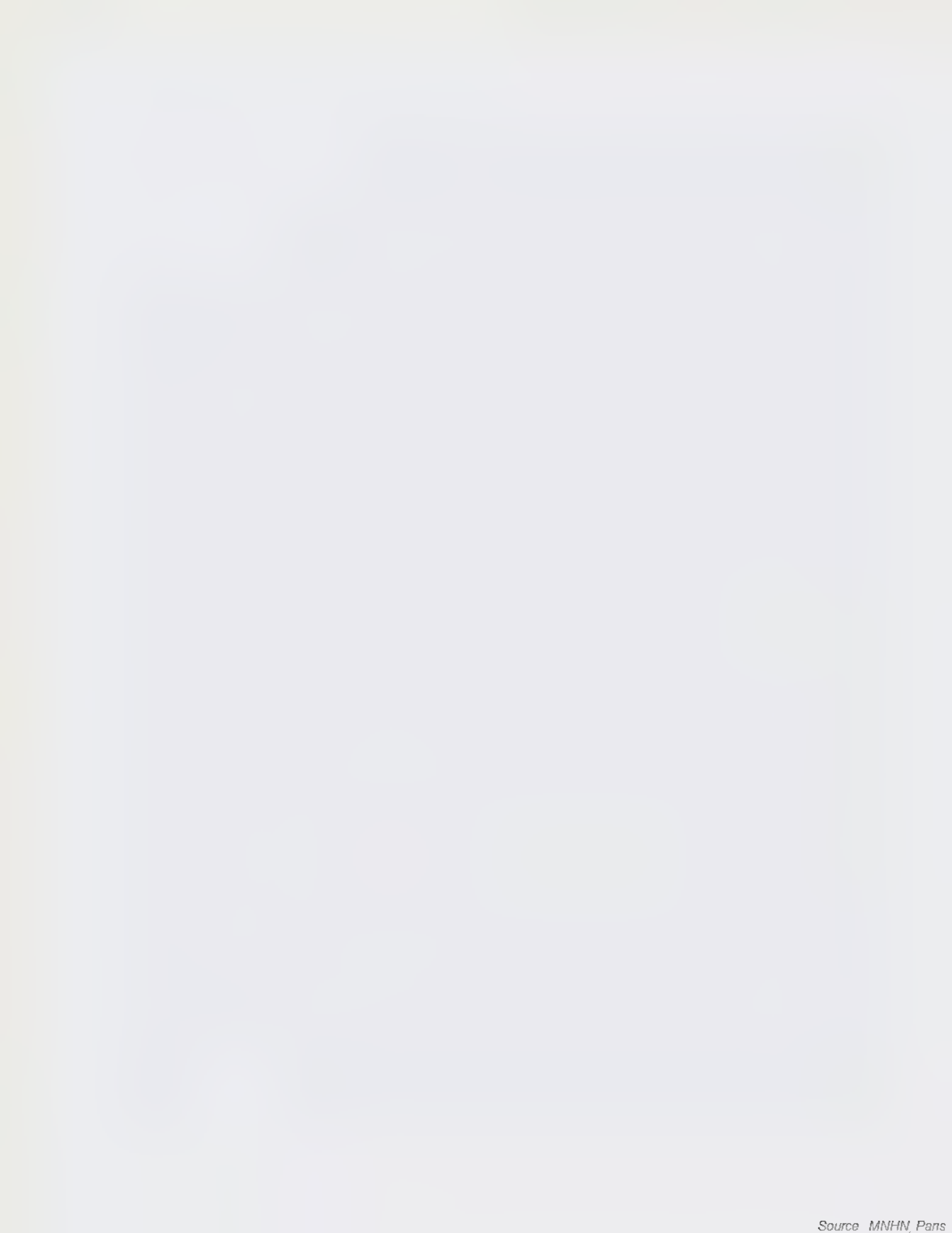

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Evolution of Feeding and Mating Behaviors in the Empidoidea (Diptera : Eremoneura)

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ABSTRACT

The phylogeny of the Empidoidea is discussed in relation to the works of ULRICH (1971), CHVALA (1983), WIEGMANN *et al* (1993), CUMMING *et al* 1995) and SINCLAIR (1995) The clade [Dolichopodidae + Microphoridae] + Tricopezinae + Brachystomatinae + Ceratomerinae] is the sister group of [Atelestidae + Hybotidae + Empididae] The family Empididae is assumed to be a monophyletic group on the basis of the presence of an endoskeletal pocket in line with the laterotergite (ULRICH, 1971, 1994), however this character is independently derived in the Tricopezinae and Ceratomerinae The phylogeny is used to interpret the evolution of two ethological characters, feeding and swarming habits Optimization of these attributes on the cladogram corroborated the hypothesis that predation is ancestral in Empidoidea Mating swarms are found to be a specialized behavior for the subfamily Empidinae and non-homologous of swarms of some other empidoids These optimizations and evolutionary pathways refute the traditional evolutionary models of swarming in the Empidoidea Finally, the hypothesis that swarming is an adaptation for the meeting of sexes is corroborated for the Empidinae

RESUME

Evolution du regime alimentaire et du comportement reproducteur chez les Empidoidea (Diptera, Eremoneura)

La phylogenie des Empidoidea est discutee en relation avec les travaux d'ULRICH (1971), CHVALA (1983) WIEGMANN *et al* (1993) et SINCLAIR (1995) Le clade des [Dolichopodidae + Microphoridae] + [Tricopezinae + Brachystomatinae + Ceratomerinae] est le groupe frere des [Atelestidae + Hybotidae + Empididae] Les Empididae sont supposes former un groupe monophyletique sur la base de la presence d'une poche endosquelettique se situant dans le prolongement du laterotergite (ULRICH 1971, 1994) cependant ce caractere est apparu par convergence chez les Tricopezinae et les Ceratomerinae La phylogenie est utilisee pour interpreter l'evolution de deux caracteres ethologiques, les comportements reproducteurs et alimentaires L'optimisation de ces attributs sur le cladogramme corrobore l'hypothese d'une origine ancestrale pour la predation chez les Empidoidea La formation d'essaims de reproduction est un comportement specialise des Empidinae qui n'est pas homologue avec les essaims formés par d'autres empidoides Ces resultats refutent les modèles traditionnels d'evolution des essaims chez les Empidoidea Par contre, l'hypothèse d'une adaptation à la rencontre des sexes pour la formation d'essaims est corroborée pour les Empidinae

INTRODUCTION

Species of Empidoidea present a large range of feeding, swarming and reproductive behaviors Many studies of these ethological characters have been carried out and several

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Source MNHN, Paris

evolutionary hypotheses have been proposed (HAMM, 1908, 1909, 1933, ELTRINGHAM, 1927, KESSEL, 1955, DOWNES, 1970, TREHEN, 1971, CHVALA, 1976) but never on a strict phylogenetic basis Nuptial gifts have been discussed by some recent authors (THORNHILL & ALCOCK, 1983) but herein I propose to study two other attributes feeding and swarming habits

Predatory habits and mating swarms have always been hypothesized as primitive habits in the Empidoidea (CHVALA, 1983), although mating swarms have only been observed in the subfamily Empidinae Other empidoids form swarms but mating behavior always takes place outside them. This point of view is traditional and deep-rooted

Recent progress in the phylogeny of the Empidoidea allows me to test traditional hypotheses of this kind with respect to phylogenetic evidence Evolutionary patterns of attributes feeding and swarming habits are inferred by mapping them on the phylogeny These inferred patterns are compared afterwards to former evolutionary hypotheses

MATERIALS AND METHODS

A character analysis for thirteen taxa and fifteen characters) was performed on the families and subfamilies of Empidoidea using the program Hennig86 (FARRIS 1988), results were analyzed with Clados, version 1 1 (NIXON, 1992) This paper does not focus on the phylogenetic tree itself, characters are thus presented in the appendix 1 Using "i e." (implicit enumeration) algorithm, a tree was obtained with the length of 21 steps, the consistency index CI – 0 80 and the retention index RI = 0 90 (Fig. 2)

The evolution of attributes was inferred by optimization on phylogenetic trees, using Fitch parsimony (FITCH, 1971) States of attributes are coded and entered in the matrix and their optimization is viewed and analyzed by Hennig86 as non-additive, under the "xx" function, without outgroup and using "ccode]" function Polymorphism between species or genera or within species in a terminal taxon is coded "?" Tree drawings were performed using Treeview (PAGE, 1996).

PHYLOGENETIC SYSTEMATICS

Historical

Among the Empidoidea (Fig 1), five families were recognized by CHVALA (1983), Empididae, Hybotidae and Microphoridae (resulting of the division of the traditional family Empididae), Atelestidae (including some genera originally classified in the Platypezidae and Hybotinae) and Dolichopodidae In the phylogeny which he proposed, the Empidoidea and Cyclorrhapha form a monophyletic group but the Atelestidae are the sister group of the Cyclorrhapha, thus the Empidoidea are paraphyletic At the present time, the Empidoidea (including the family Atelestidae) are recognized as a monophyletic taxon supported by four synapomorphies (CUMMING *et al.*, 1995) and sister group of the Cyclorrhapha, forming together the clade Eremoneura supported by ten synapomorphies (MCALPINE, 1989, SINCLAIR, 1992, CUMMING *et al.*, 1995)

WIEGMANN *et al.* (1993) proposed some phylogenetic hypotheses and focused on the theories (epandrial and periandrial) of male genitalia evolution Unfortunately, as SINCLAIR (1995 719) noted, WIEGMANN's hypotheses are poorly supported, several characters being highly polymorphic and the others incorrectly scored

SINCLAIR (1995) reduced the family Empididae (*sensu* CHVALA) to four subfamilies, Empidinae, Hemerodromiinae, Clinocerinae and Oreogetoninae The Brachystomatinae, Ceratomerinae and Tricopezinae (The subfamily Tricopezinae was newly defined by SINCLAIR, 1995, its monophyly is supported by the presence of a median apodeme in the female

Source MNHN, Paris

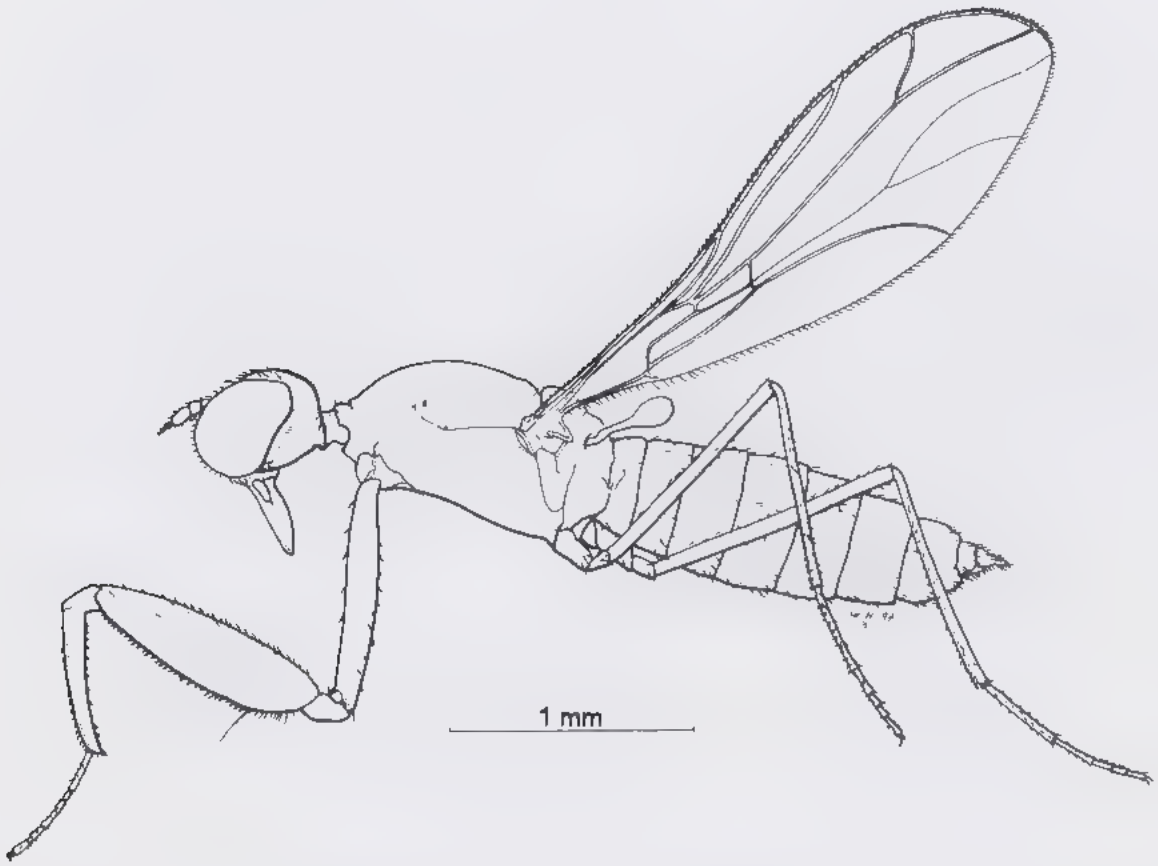

FIG. 1. — *Hemerodromia sp* (from French Guiana), female, habitus. Scale bar = 1 mm

postabdomen) are hypothesized to be a monophyletic taxon, sister group of [Dolichopodidae + Microphoridae] on the basis of several characters of female postabdomen largely detailed by SINCLAIR (1995: 718-719, characters 1, 2, 3 and 4, see also appendix 1, characters 11-14)

Characters

The works of ULRICH (1971), CHVALA (1983), WIEGMANN *et al.* (1993), CUMMING *et al.* (1995) and SINCLAIR (1995) have been re-analyzed to propose a matrix of 15 morphological characters (Appendix 1) The principal subject of this paper is not the phylogeny of the Empidoidea but the evolution of ethological characters, I refer readers to the original references for more details about the morphological characters Nevertheless, at this point it is pertinent to comment of two of the characters

Prokatepisternum fused with the basisternite (Character 3) Primitively, the basisternite (= prosternum), a sclerite located between the front coxae, is small and isolated, but in numerous taxa of Diptera it is developed laterally and fused with the anterior ventral episternum

Source MNHN, Paris

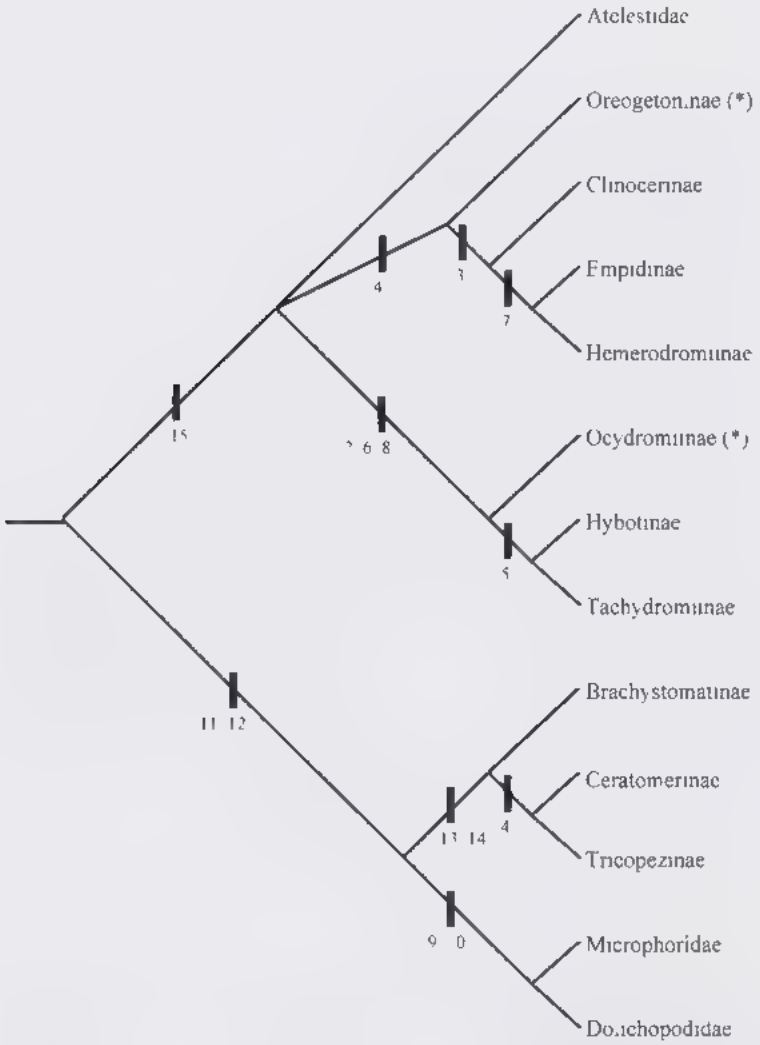

FIG 2 Cladogram of the families and subfamilies of Empidoidea, according to data presented in appendix 1 and treated as described in materials and methods * paraphyletic taxon

(prokatepisternum) to form a precoxal bridge (SPEIGHT, 1969) This has been observed in the Empidinae, Hemerodromiinae, Clinocerinae, Brachystomatinae, Ceratomerinae, some Tricopezinae (at least *Heterophlebus*, *Hyperperacera* and *Heleodromia*), some Dolichopodidae and some Tachydromiinae

Presence of an endoskeletal ridge in mesanepimeron (Character 4) ULRICH (1971) showed the existence of an endoskeletal ridge in mesanepimeron in some empidoids, forming a characteristic complete or incomplete pocket in line with the laterotergite It is curious that this character has never been used or commented by other workers since 1971 A complete pocket has been observed in the Oreogetoninae (ULRICH, 1994), Clinocerinae (*Wiedemannia*, *Dolichocephala*, ULRICH, 1971), Hemerodromiinae (*Chelipoda*, ULRICH, 1971), Tricopezinae (*Tricopeza*, *Rubistella*, ULRICH, 1971, 1994) and Ceratomerinae (ULRICH, 1994), whereas an incomplete pocket seems present in all species of the subfamily Empidinae, and some

Source MNHN, Paris

Tricopezinae (at least *Heterophlebus*) Consequently this character has been used in the matrix (Appendix 1) under 3 states and treated as non-additive

Phylogeny: Empididae as a monophyletic group (Fig. 2)

The monophyly of the Empididae is supported by the presence of an endoskeletal ridge in the mesanepimeron forming a complete pocket in line with the laterotergite even if this pocket is secondarily reduced dorsally (Empidinae) or entirely lost in the more specialized Hemerodromiinae like *Chelifera* where the lengthening of thorax has led to the loss of pleural sutures It is hypothesized that the presence of the pocket is a synapomorphy for the clade [Oreogetoninae + Clinocerinae + Hemerodromiinae + Empidinae] A pocket of the same structure and position existing in Tricopezinae and Ceratomerinae, it is provisionally supposed that these two taxa are sister groups

Within the clade [Atelestidae + Hybotidae + Empididae], supposed monophyletic on the basis of the absence of acanthophorites in the female (SINCLAIR, 1995), only the Clinocerinae, Hemerodromiinae and Empidinae (and also a few species of Tachydromiinae) possess a precoxal bridge Consequently, these three subfamilies form a monophyletic group within the Empididae A precoxal bridge has appeared independently once or several times in the clade [Dolichopodidae + Microphoridae + Ceratomerinae + Brachystomatinae + Tricopezinae]

THE ATTRIBUTES

Feeding habits

Most Empidoidea are predators, however some are also flower visitors (pollen or nectar feeders) Table 1 shows the different feeding habits observed in the superfamily Two subfamilies

TABLE 1. — Feeding habits in the Empidoidea. A question mark indicates an unknown state

Taxa	Predators	Nectar or pollen feeders
Atelestidae	?	?
Oreogetoninae	in flight	x
Clinocerinae	in flight	
Empidinae	in flight	x
Hemerodromiinae	on solid substratum	
Ocydromiinae	in flight	x
Hybotinae	in flight	
Tachydromiinae	on solid substratum	
Brachystomatinae	?	?
Ceratomerinae	?	?
Tricopezinae	?	?
Microphoridae	in flight	x
Dolichopodidae	in flight	x

Source MNHN Paris

of the Empididae (Clinocerinae and Hemerodromiinae) as well as two subfamilies of the Hybotidae (Hybotinae and Tachydromiinae) may be considered as entirely predatory, the remaining families and subfamilies are both predators and pollen or nectar feeders The Dolichopodidae are entirely predators apart from the genus *Hercostomus* of which species are nectar feeders (LAURENCE, 1953) The Microphoridae (genus *Microphorus*) are generally predators but also often found on flowers (CHVALA, 1983) but we do not know if they are pollen or nectar feeders The Hybotidae genera *Anthalia*, *Allanthalia* and *Euthyneura* are pollen or nectar feeders but one species (at least), *Anthalia bulbosa*, is known to feed on pollen (DOWNES & SMITH, 1969), the remaining Hybotidae are entirely predators (CHVALA, 1983). The Oreogetoninae genera *Iteaphila* and *Anthepiscopus* are pollen or nectar feeders whereas *Hormopeza* species are predators (CHANDLER, 1972) The Empidinae are nectar feeders but during the mating period males hunt other insects which are offered to females as a nuptial gift Three remarks are necessary, (1) males never feed preys which they have caught, (2) in the genus *Hilara*, the gift can be a simple vegetal fragment not edible to the female (TREHEN, 1965), (3) species of this genus have rarely been observed outside the habitat (generally places with presence of water: river, lake or simple puddle) where individuals hunt and mate, consequently evidence of their feeding habits is lacking The feeding habits of Tricopezinae, Ceratomerinae, Brachystomatinae and Atelestidae are almost entirely unknown on account of the scarcity of their species in the nature CHVALA (1983) supposed that the Ceratomerinae are flower visitors because their proboscis is elongated, but the presence of a morphological character is not unequivocal evidence of the existence of a behavior

Among the Empidoidea, it is possible to distinguish four chief classes, species entirely predators (Hybotinae, Tachydromiinae, Clinocerinae and Hemerodromiinae), species entirely flower visitors (*Hercostomus*, *Anthalia*, *Allanthalia* and *Euthyneura*), species both predators and flower visitors (*Microphorus*) and the flower visiting species in which predation is only performed by males and during the mating period (Empidinae) Among the predators, the hunting can take place in flight (Dolichopodidae, Microphoridae, Hybotinae, Ocydromiinae, Oreogetoninae, Clinocerinae and Empidinae) or on the ground (Tachydromiinae and Hemerodromiinae).

Swarming habits

Swarming is a well known habit in many groups of Diptera (GRUHL, 1955, MCALPINE & MUNROE, 1968, DOWNES, 1969, CHVALA, 1990) especially in the Empidoidea Indeed, 5 families or subfamilies of empidoids display this behavior, the Microphoridae, Atelestidae, Ocydromiinae, Oreogetoninae and Empidinae The remaining Empidoidea have never been observed to form swarms (Dolichopodidae, Hybotinae, Tachydromiinae, Clinocerinae and Hemerodromiinae) or are insufficiently known (Brachystomatinae, Ceratomerinae and Tricopezinae)

GRUHL (1955) distinguished synhesmic swarming from synorchesic swarming The first is characterized by an unorganized mass of a large number of individuals resulting from mass emergence, the second forms coherent units characterized by an ordered flight (often species-specific) of several individuals termed true synorchesia Gruhl also distinguished several evolutionary steps leading to this true synorchesia, chiefly the prosynorchesium (a pursuit flight of males from their perching places), the monorchesium (the hovering and dancing of isolated individuals) and the polyorchesium (the rhythmic alternation of dancing-perching-dancing of a

Source MNHN, Paris

few individuals) Among the Empidoidea, it seems that the Empidinae form the polyorchesium swarms, whereas the other groups, especially Ocydromiinae, have generally observed in monorchesium swarming (CHVALA, 1983).

Swarming in the Empidoidea has always been linked to the meeting of sexes and thus to the mating In fact, as CHVALA (1980) noted, it seems that this is characteristic of the Empidinae only Indeed, in this group, males are seen in swarms with prey that are offered to females just before mating Mating begins in the swarm and ends on a solid substratum In the other Empidoidea forming swarms, hunting or mating have never been observed in swarms

It seems therefore that, both structurally and functionally, swarms formed by the Empidinae are distinguishable from these of the Atelestidae, Microphoridae, Oreogetoninae and Ocydromiinae That is why in table 2 we consider 3 cases among the superfamily, groups of which species never form swarms (no swarming activity), these for which swarming represents a monorchesium and in which hunting or mating has not been observed (swarms without mating), and these for which swarming represents a polyorchesium or true synorchesia and which are linked with both a predatory and mating activities (mating swarms)

Nevertheless, we will also consider the case where two attributes under two states are successively treated, presence and absence of swarms and beginning of mating in or outside swarms

TABLE 2 — Swarming and mating habits in the Empidoidea. A question mark indicates an unknown state

Taxa	No swarming activity	swarms without mating	Mating swarms
Atelestidae		x	
Oreogetoninae		x	
Clinocerinae	x		
Empidinae			x
Hemerodromiinae	x		
Ocydromiinae		x	
Hybotinae	x		
Tachydromiinae	x		
Brachystomatinae	?	?	?
Ceratomerinae	?	?	?
Tricopezinae	?	?	?
Microphoridae		x	
Dolichopodidae	x		

FORMER EVOLUTIONARY HYPOTHESES

Feeding habits

Predatory activity has always been considered ancestral for the Empidoidea (CHVALA, 1983) for at least three reasons, (1) it is a very widespread habit in the superfamily, the flower visitor species being only found in a few genera and one subfamily, (2) predation is considered the basal feeding habit of the Asiloidea and Empidoidea, (3) oldest known fossil of Empidoidea is

Source MNHN, Paris

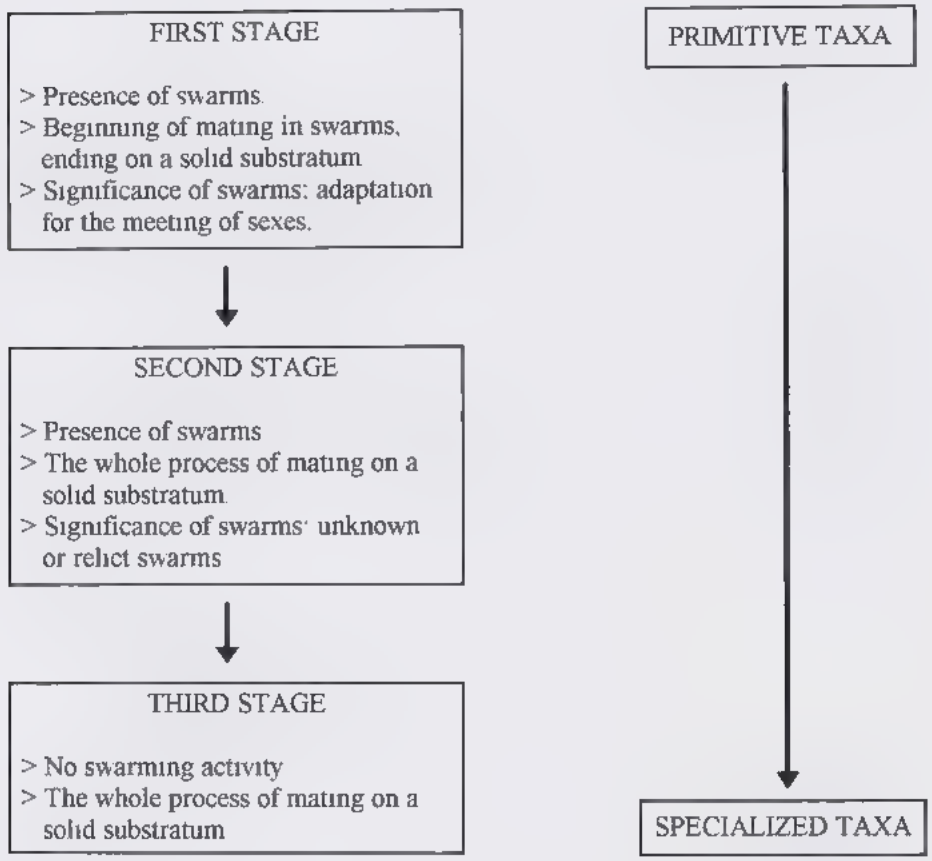

FIG 3 Model of the evolution of swarming in Diptera (after MCALPINE & MUNROE, 1968, DOWNES, 1969, CHVALA, 1983)

dated from 160 millions years (middle Jurassic) (USACHEV, 1968) before the rise of the angiosperms.

The fact that predation is very widespread in the superfamily is not evidence of its supposed ancestral origin. Such hypothesis can be compared to the ingroup distribution criteria used in phylogeny to establish the polarity of characters. Now we know that this criteria is not valuable. On the other hand, in the Asiloidea, only the Asilidae are predatory, and the habits of the Asiloidea could be informative in this context if the Empidoidea and Asiloidea are sister groups, but the sister-group of the Empidoidea is the Cyclorrhapha (CUMMING *et al.*, 1995).

For these reasons, it seems justified to test the hypothesis of plesiomorphy of predation for the Empidoidea. We will also test the hypothesis that predation in flight is ancestral to predation on the ground.

Source: MNHN, Paris

Swarming habits

The significance and evolution of swarms in the Diptera have been studied in several papers, GRUHL (1955), MCALPINE & MUNROE (1968), DOWNES (1969) and CHVALA (1976, 1980, 1990) From these works, we can present consensus which may be summarized in two points, (1) swarming is ancestral for the Diptera in general and each taxon of Diptera which presents this behavior in particular, (2) the original function of swarming is the meeting of sexes, whereas the mating or hunting on a solid substratum is a specialized activity This consensus is the result of two observations which have been detailed by MCALPINE & MUNROE, (1) swarming exists in the main lineages of Diptera, nevertheless being more widespread in the Nematocera (reputed primitive) than in the Brachycera (reputed specialized), (2) for Diptera in particular and insects in general which present a swarming activity, the meeting of sexes and the beginning of mating often take place in the swarms To summarize these hypotheses (MCALPINE & MUNROE,1968 1167) "The remarkable correlation between swarming and mating habits [ ] in the phyletically distant Diptera, is a clear indication that swarming and coupling in flight are basic to a dipterous condition".

Nevertheless, there exists a large number of Diptera forming swarms of which the function remains unknown As presented earlier, no swarming Empidoidea has been observed to mate in the swarms except species of the subfamily Empidinae Several unconvincing hypotheses have been proposed to explain the significance of these swarms (for example see PAJUNEN, 1980) CHVALA (1983) proposed that these swarms are relicts of an ancestral behavior in which mating is correlated with swarming, mating activity of these groups would have been transferred on a solid substratum and swarms would be without particular significance

These hypotheses can be summarized in an evolutionary model (Fig 3) that is applies to the whole order Diptera Among the Empidoidea, families and subfamilies can be affiliated to one stage of model (first stage) Empidinae meeting of sexes and beginning of mating in the swarm, swarms are an adaptation for the meeting of sexes, (second stage) Atelestidae, Oreogetoninae, Ocydromiinae and Microphoridae meeting of sexes and mating transferred on a solid substratum, swarms as relict of ancestral behavior, (third stage) Dolichopodidae, Hybotinae, Tachydromiinae, Clinocerinae and Hemerodromiinae meeting of sexes and mating on a solid substratum, loss of the ability to form swarms

THE TESTS OF EVOLUTIONARY HYPOTHESES

The reconstruction of phylogenies is independent from most process theories (ELDREDGE & CRACRAFT, 1980), that is why the optimization method, as described by BROOKS & MCLENNAN (1991) among others, allows evolutionary models to be tested objectively The optimization consists of mapping the previously defined attributes on the phylogeny and follow their evolution on the cladogram with respect of the parsimony principle

Feeding habits

Predator Flower visitor Feeding habits were scored in two states, predator or flower visitor After optimization, the cladogram (Fig 4) shows that predation is plesiomorphic for all the Empidoidea but also for the clades [Dolichopodidae + Microphoridae], Hybotidae and Empididae The flower visiting habit is apomorphic for the Empidinae, predation having only been conserved during the mating period The flower visiting habit has appeared independently in

Source MNHN, Paris

several other lineages of Empidoidea, but two cases must be distinguished The first is represented by the Dolichopodidae, Ocydromiinae and Oreogetoninae, for which some genera became flower visitors (see the chapter "attributes"), and the second is represented by species of the genus *Microphorus* (Microphoridae) which are both predators and flower visitors It is possible to consider that the flower visiting habit has recently appeared in this family The traditional model is therefore corroborated by the phylogenetic test.

Predation in flight Predation on solid substratum The attribute (predation) has been considered under two states (in flight or on solid substratum) After optimization, the phylogeny (Fig 5) shows that predation in flight is ancestral for all the Empidoidea and the clades [Dolichopodidae + Microphoridae], Hybotidae and Empididae Even if predation in flight is plesiomorphic, it must be noted that this is considerably diversified and specialized in present taxa, for instance females of some microphorids of the genus *Microphorus* catch other insects in the spider webs, the Clinocerinae and males of the genus *Hilara* (Empidinae) hunt on the surface of water, the second of these wrap up prey in a silk balloon which is presented to the females just before mating as a nuptial gift To summarize, different forms of hunting in flight observed among the Empidoidea are apomorphic for each considered taxon

Hunting on a solid substratum (on the ground or on a leaf for example) is apomorphic for the Tachydromiinae (Hybotidae) and Hemerodromiinae (Empididae) (Fig 5) Thus it is a very specialized type of predation that appeared by convergence in these two subfamilies Species in these two subfamilies also possess a convergently specialized morphology where the thorax is elongated and the fore and sometimes middle legs are raptorial with elongated coxae, thick femora, and bent tibiae (Fig 1) These morphological characters are likely to be an adaptation for predation on a solid substratum because Hemerodromiinae and Tachydrominae are respectively the only taxa in Empididae and Hybotidae to present them Tachydromiinae and Hemerodromiinae are therefore a remarkable example of both morphological and behavioral convergences Of course, this does not mean that the presence of such characters involves such behaviors, unfortunately this "rule" is often applied in evolutionary biology and cases of exaptation are often overlooked

Swarming habits

Presence Absence of swarms According to optimization, two equally parsimonious patterns (4 steps) (Figs 6, 7) have been obtained In both cases, swarming is plesiomorphic for all the Empidoidea, [Dolichopodidae + Micophoridae], [Atelestidae + Hybotidae + Empididae], and for the Hybotidae and Empididae themselves, whereas the absence of swarming is apomorphic for the Dolichopodidae and [Hybotinae + Tachydromiinae] In the Empididae, the situation is more complex because, although in the first and second patterns, swarming appears as ancestral for the family, two cases must be considered for the clade [Clinocerinae + Empidinae + Hemerodromiinae] In the first pattern (Fig 6), swarming is plesiomorphic for this clade but also the [Empidinae + Hemerodromiinae], the loss of this behavior occurring twice, once in the Clinocerinae and a second time in the Hemerodromiinae Swarming remains therefore plesiomorphic for the Empidinae whereas the absence of swarming is apomorphic for the Clinocerinae and Hemerodrominae In the second pattern (Fig 7), the absence of swarming is ancestral for the [Clinocerinae + Empidinae + Hemerodromiinae] with a reversion for the Empidinae, swarming being therefore apomorphic for this subfamily

Source MNHN Paris

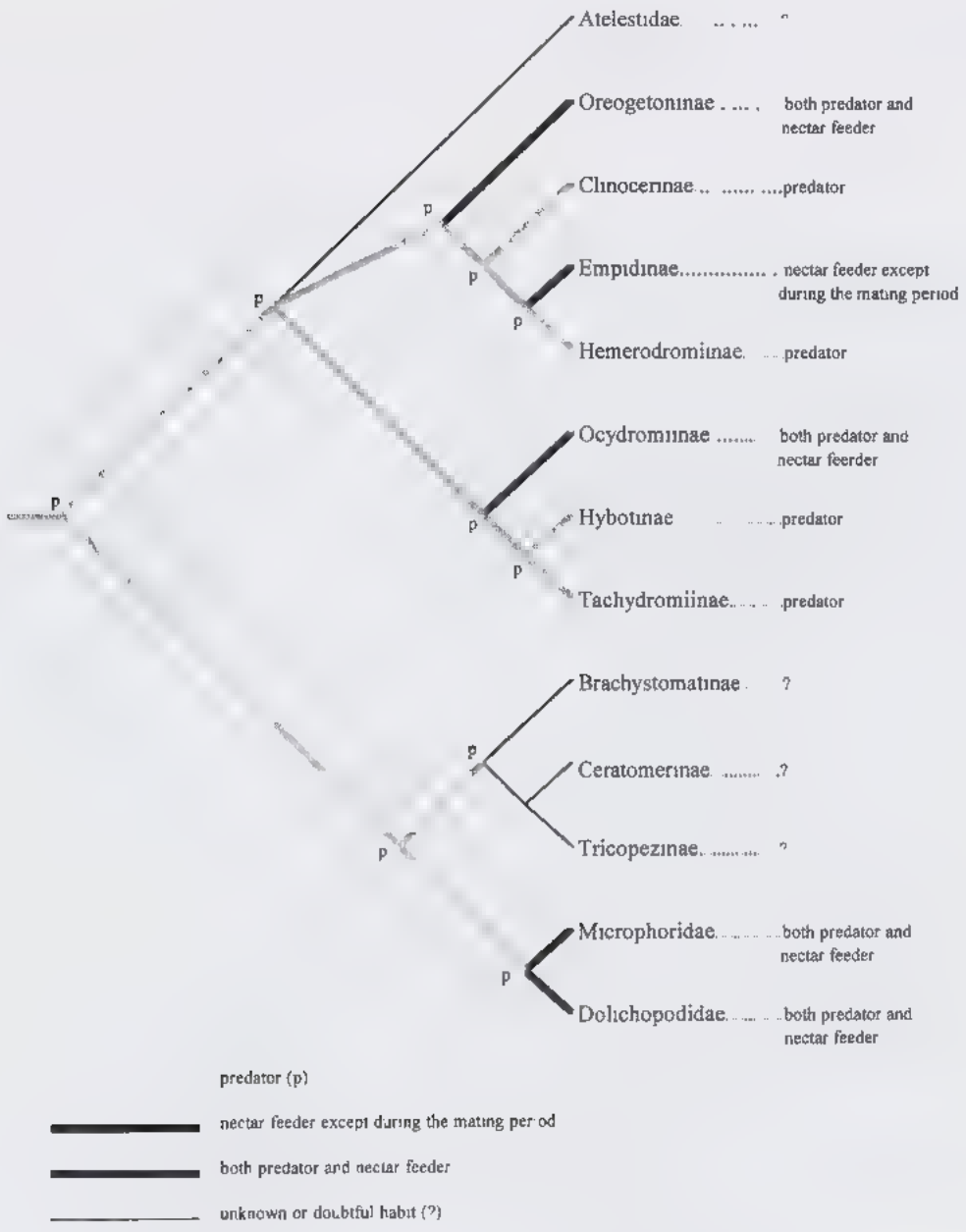

FIG 4 — Cladogram of Empidoidea showing optimization of feeding habits

Mating in outside swarms Mating in swarms being specific of Empidinae, this behavior is apomorphic for this group (Fig 8) Conversely mating outside swarms is plesiomorphic for all the Empidoidea and all clades of the phylogeny including the terminal taxa apart from, of course, the Empidinae

Swarming in Empidinae: exaptation or adaptation to the meeting of sexes? The cladistic tests of adaptation hypotheses have been reviewed by CODDINGTON (1988) and GRANDCOLAS *et*

Source MNHN, Paris

FIG 5 — Cladogram of Empidoidea showing optimization of type of predation

al. (1994) To summarize, a character is an adaptation for a given taxon if it has appeared in this taxon with the additional assumption of its selective value

If we combine the previous result (mating in swarms as apomorphic for the Empidinae) with both equally parsimonious patterns for the first attribute (presence or absence of swarms) then two cases are possible for the Empidinae, (1) swarming is plesiomorphic and has been inherited from the ancestor of the Empidoidea (and swarms formed by different Empidoidea are homologous) for which the phylogeny shows mating did not take place in the swarms (Fig 6)

Source MNHN Paris

They therefore had a different function that was not to allow the meeting of sexes Consequently, swarming in the Empidinae is an exaptation (GOULD & VRBA, 1982) to the meeting of sexes (2) Swarming is apomorphic for the Empidinae (Fig 7) and it appeared with the subfamily (swarms formed by the Empidinae are therefore not homologous with these formed by some other Empidoidea) The mating in swarms arising in the Empidinae, the adaptive hypothesis that swarming evolved for the meeting of sexes is corroborated by the phylogeny

To summarize (Table 3): according to both patterns, the traditional model is refuted in two points, (1) the ancestral state is not the formation of mating swarms but the formation of swarms of which the function is unknown, the mating taking place on a solid substratum (or may be in flight but not in swarm), (2) mating in swarms is an apomorphic behavior that appeared with the Empidinae

In addition, the pattern 1 refutes the adaptation hypothesis of swarming for the meeting of sexes in Empidinae whereas the pattern 2 corroborates it Finally, the absence of swarms as apomorphic character is corroborated for Dolichopodidae and [Hybotinae + Tachydromiinae] by both patterns and Hemerodromiinae and Clinocerinae by the first pattern but refuted by the second because in this case the loss of the ability to form swarms took place for the ancestor of the clade [Clinocerinae + Empidinae + Hemerodromiinae]

Is it possible to choose between exaptation and adaptation for the formation of swarms in the Empidinae? In fact we can only consider one attribute (swarming) under three states (absence swarming without mating mating swarms) rather than two attributes (swarming · mating)

TABLE 3 Former evolutionary hypotheses and their tests by two phylogenetic patterns (optimizations on the cladogram) * in the empidids, the corroboration is only true for the clade [Clinocerinae + Empidinae + Hemerodromiinae] with a reversion for the Empidinae

Former evolutionary hypotheses	According to pattern 1	According to pattern 2
Mating swarms . plesiomorphic	Refuted	Refuted
Swarms without mating relict from mating swarms	Refuted	Refuted
Mating swarms· adaptation to the meeting of sexes (in Empidinae)	Refuted	Corroborated
No swarming activity apomorphic	Corroborated	Corroborated *

under two states (respectively absence presence and in or outside swarms) for the reasons explained in the chapter "Attributes"

Absence of swarms swarming without mating mating swarms After optimization, a pattern (Fig 9) as parsimonious (4 steps) as the previous ones has been found Swarming

Source MNHN Paris

FIG 6. — Cladogram of Empidoidea showing optimization of swarming habits. First pattern

without mating is plesiomorphic for the Empidoidea, [Dolichopodidae + Microphoridae], Atelestidae, Hybotidae and Empididae, the absence of swarms is apomorphic for the Dolichopodidae, [Hybotinae + Tachydromiinae] and [Clinocerinae + Empidinae + Hemerodrominae] On the other hand, the Empidinae are the only Empidoidea which form mating swarms This behavior is therefore apomorphic for this subfamily and as in the pattern 2, the adaptation hypothesis for swarming to the meeting of sexes is corroborated.

Source MNHN Paris

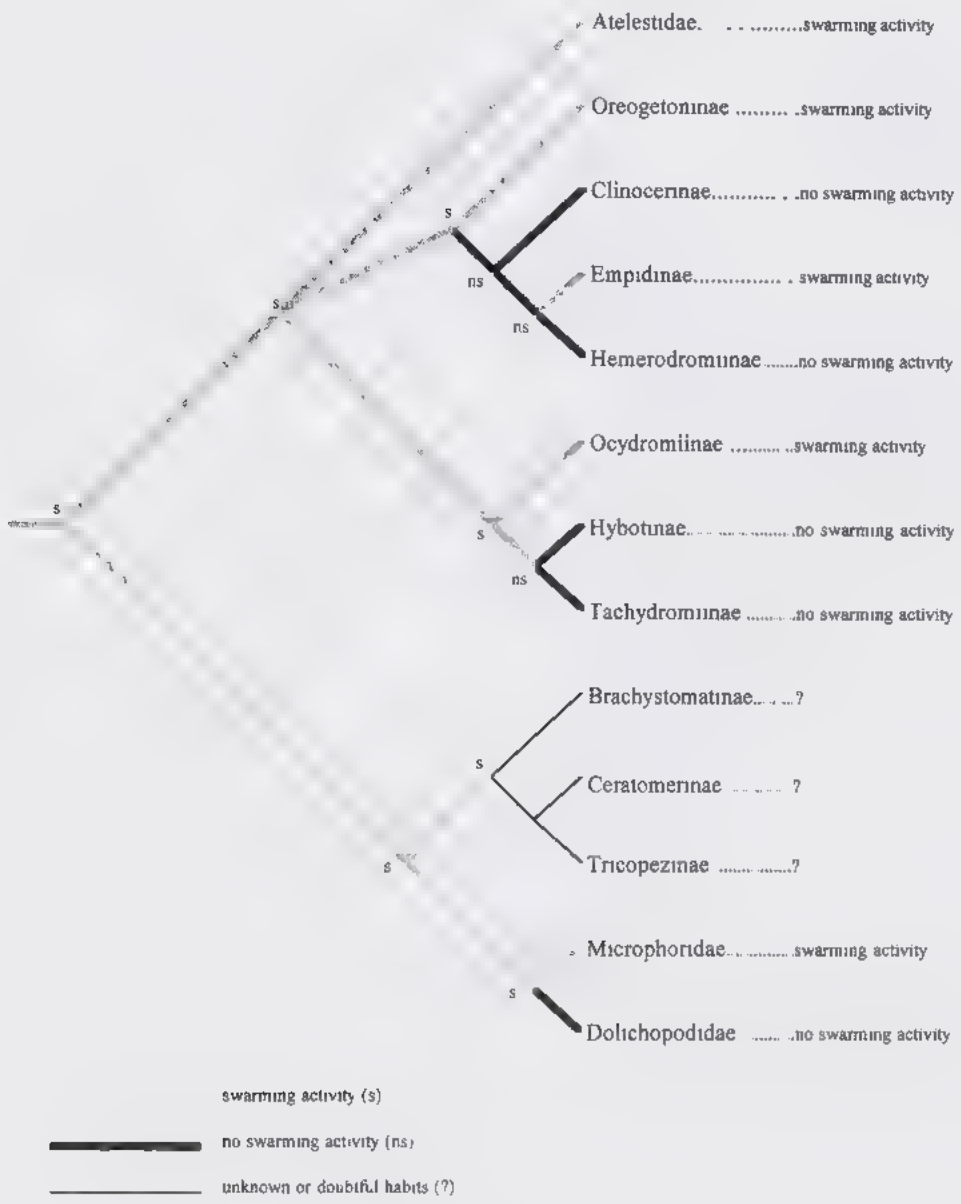

FIG 7. — Cladogram of Empidoidea showing optimization of swarming habits Second pattern

DISCUSSION

Swarms formed by the Empidinae are structurally (according to the GRUHL classification, 1955) different from those formed by some other Empidoidea Within these swarms, the Empidinae show a succession of behaviors (hunting, meeting of sexes, nuptial gift, beginning of mating) never observed in any other Empidoidea In the Empidinae, swarming and mating seem therefore correlated, this pleads in favor of the consideration of one attribute under three states (no swarming activity, swarming without mating and mating swarms) Nevertheless the

Source MNHN Paris

FIG. 8. — Cladogram of Empidoidea showing optimization of type of mating.

consideration of two attributes allows two patterns of which one gives results analogous with those obtained with one attribute Consequently it seems that the following conclusions force themselves

1) Swarming and mating outside swarms are plesiomorphic for the Empidoidea

2) The absence of swarms in some Empidoidea is apomorphic for three clades, Dolichopodidae, [Hybotinae + Tachydromiinae] and [Clinocerinae + Empidinae + Hemerodromiinae]

Source MNHN Paris

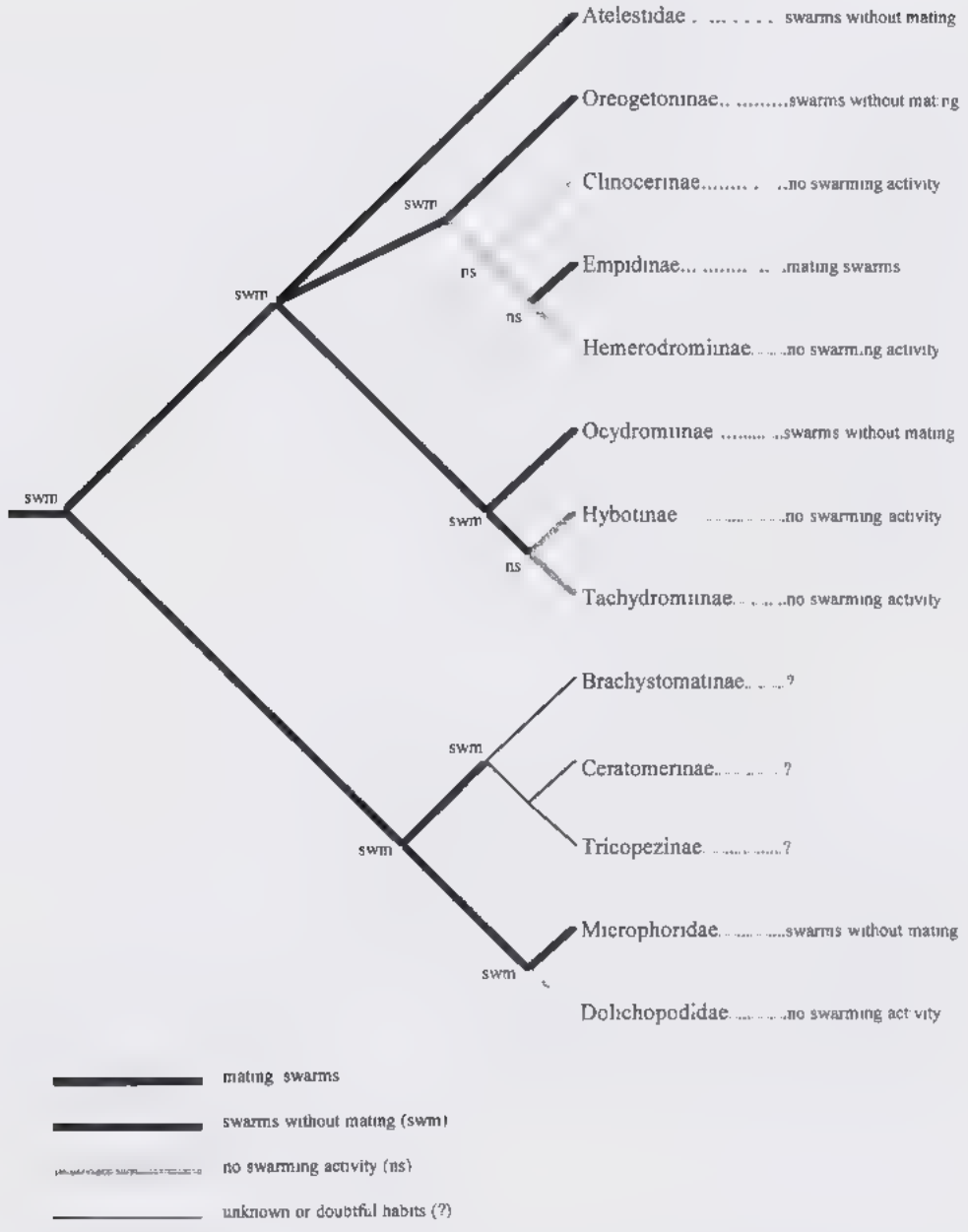

FIG 9. — Cladogram of Empidoidea showing optimization of both swarming and mating.

3) Mating swarms are apomorphic for the Empidinae but not homologous with swarms formed by other Empidoidea. Thus they cannot be integrated to a global evolutionary model and thus do not form the basis of this model as it has always been presented. On the other hand, in the Empidoidea, swarming without mating cannot be considered a relict from mating swarms.

4) The adaptation hypothesis of swarming to the meeting of sexes is corroborated for the Empidinae without, of course, prejudging of the selective value of this behavior.

Source: MNHN, Paris

CONCLUSION

The optimization of characters on the phylogeny leads to refutable results and is the only objective test of evolutionary models because of its independence from these models Thus systematics must not be only considered as the science of inventories, descriptions and classifications of taxa in predictive systems, but also as the science of explanatory framework for character evolution

This method is therefore employed herein for the first time for the Empidoidea, but important advances remain to be achieved in both phylogeny and ethology of the Empidoidea before to offer more stable evolutionary hypotheses

Finally, focusing on the phylogenetic patterns in the subfamily Empidinae is probably the most interesting perspective CUMMING (1994) proposed an evolutionary model for this group with reference to sexual selection Using phylogeny, it will be therefore possible to test this model and other hypotheses relating the origin and evolution of both nuptial gift and female ornementations

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Source: MNHN, Paris

APPENDIX 1 — List of characters and Matrix. In brackets, bold-faced and italic types indicate respectively the number of the page and the number of the character in the original work.

1 Maxillary lacinia absent: 1, present: 0 (CHVALA, 1983 **61**, *12*, WIEGMANN *et al.*, 1993 **66**, *9*)
2 Palpi connected to palpifer: 1, attached to stipites: 0 (CHVALA, 1983 **61**, *13*, WIEGMANN *et al.*, 1993 **66**, *8*).
3 Prokatepisternum fused with the basisternite: 1, isolated: 0 (CHVALA, 1983 **61**, *10*; WIEGMANN *et al.*, 1993 **66**, *12*)
4 Mesoanepimeral suture present and forming a ¼ - half of circle: 1, present and forming a ¼ of circle: absent: 0 (ULRICH, 1971, *Figs 3*, *4*, *5*, *44*, *45*, 1994 **230**)
5 Two discal veins: 1, three discal veins: 0 (CHVALA, 1983 **61**, *8*, WIEGMANN *et al.*, 1993 **68**, *21*)
6 Front tibiae with tubular gland: 1, tubular gland absent: 0 (CHVALA, 1983 **61**, *11*, WIEGMANN *et al.* 1993 **70** *28*)
7. male cerci sclerotized: 1, not sclerotized: 0 (ULRICH, 1975, CUMMING *et al.*, 1995 **133**, *12*)
8 Rotation of male hypopygium between 45°and 90°: 1 Hypopygium without rotation: 0 (CHVALA, 1983 **61**, *3*, CUMMING *et al.*, 1995 **133**, *14*)
9 Rotation of both male hypopygium and segments 7 and 8: 1, Hypopygium and segments 7 and 8 without rotation: 0 (CUMMING *et al.*, 1995, **133**, *15*)
10 Bacilliform sclerite and hypandrium fused: 1, no fused: 0 (CUMMING *et al.*, 1995 **134**, *16*).
11 Sternite 8 of female articulated or fused with tergite 8: 1, well separated: 0 (SINCLAIR, 1995 **718**, *1*).
12 female cerci sclerotized: 1, not sclerotized: 0 (SINCLAIR, 1995 **718**, *2*)
13 Tergite 7 of female with a fringe of bristles on the posterior margin: 1, without fringe of bristles: 0 (SINCLAIR, 1995 **719**, *3*)
14 female cerci upright: 1, horizontal: 0 (SINCLAIR, 1995 **719**, *4*).
15 Acanthophorites absent: 1, present: 0 (SINCLAIR, 1995: **668**)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Atelestidae	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Oreogetoninae	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
Clinocerinae	0	0	1	1	0/1	0	0	0	0	0	0	0	0	0	1
Empidinae	0	0	1	1	0	0	1	0	0	0	0	0	0	0	1
Hemerodromiinae	0	0	1	1	0/1	0	1	0	0	0	0	0	0	0	1
Ocydromiinae	1	1	0	0	0	1	0	1	0	0	0	0	0	0	1
Hybotinae	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1
Tachydromiinae	1	1	0/1	0	1	1	0	1	0	0	0	0	0	0	1
Brachystomatinae	0	0	1	0	0	0	0	0	0	0	1	1	1	1	0
Ceratomerinae	0	0	1	1	0	0	0	0	0	0	1	1	1	1	0
Tricopezinae	0	0	0/1	1	0	0	0	0	0	0	1	1	1	1	0
Microphoridae	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0
Dolichopodidae	0	0	0/1	0	1	0	0	0	1	1	1	1	0	0	0

Source: MNHN, Paris

Acoustic Communication in Crickets (Orthoptera: Grylloidea): A Model of Regressive Evolution Revisited Using Phylogeny

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ABSTRACT

Acoustic communication is essential in cricket biology, being related to mating behavior. Current hypotheses on the evolution of acoustic communication in crickets consider that singing is ancestral in crickets, and that it has been lost several times in different cricket lineages. According to studies of cricket populations, it has also been hypothesized that the loss of acoustic communication could have occurred following a progressive transformation series. Similarly, it has been assumed that several factors could have influenced that evolution: such as predation pressure, low efficiency of acoustic communication due to poor environmental conditions, an evolutionary shift toward another mode of communication, population structure or habitat of the taxa. I present a phylogenetic test of this model. Song characteristics were optimized onto the phylogenetic trees for two clades of cricket (Grylloidea, Phalangopsidae) and the resultant phylogenetic patterns compared with the theoretical patterns implied by the pre-existing hypotheses. My study produced four main results: (1) multiple and convergent absences of songs occurred, (2) no linear and progressive transformation series toward complete song loss was found, (3) the polarization of the presence/absence of songs was not always in the sequence predicted by the model, (4) reversals from song lack to song presence were documented. Such reversals have never been hypothesized before, and the acoustic evolution of crickets appeared highly homoplastic. Phylogenetic analyses showed that factors such as predation pressure, population structure, etc., cannot be characterized on the basis of their definite evolutionary effect on acoustic communication: consequently previous hypotheses on their possible influence on cricket evolution cannot be tested. Although many papers have been written on acoustic communication in crickets, no clear and general hypothesis yet exists for its origin and evolution. Integrated studies of both phylogeny and population biology are badly needed to generalize the results presented in this paper, and to support new hypotheses on the subject.

RÉSUMÉ

La communication acoustique chez les Grillons : un modèle d'évolution régressive testé à l'aide de la phylogénie

La communication acoustique occupe une place importante dans la biologie des grillons, principalement dans le contexte de la reproduction. Les hypothèses classiques sur l'évolution acoustique des grillons considèrent que ce mode de communication leur est ancestral et qu'il a été perdu au cours de l'évolution à de multiples reprises et de manière indépendante. A partir d'études de populations, un modèle d'évolution a ainsi été proposé, selon lequel la communication acoustique aurait été perdue à plusieurs reprises de manière progressive, suivant des étapes bien définies. Parallèlement, des hypothèses ont été émises sur les facteurs susceptibles d'influencer l'évolution acoustique chez les grillons (prédation, efficacité de ce mode de communication dans le milieu ambiant, habitat, structure des populations, évolution vers un autre mode de communication).

DESUTTER-GRANDCOLAS, L. 1997. Acoustic communication in crickets (Orthoptera: Grylloidea): A model of regressive evolution revisited using phylogeny. *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mem. Mus. natn. Hist. nat.*, **173**: 183-202. Paris. ISBN 2-85653-508-9

Source: MNHN, Paris

Un test phylogénétique de ces hypothèses est présenté, à partir des analyses phylogénétiques de deux clades de grillons (Grylloidea Phalangopsidae) Les patterns phylogénétiques obtenus par optimisation des chants sur la phylogénie de ces deux clades sont comparés aux patterns théoriques dérivés des hypothèses testées L'hypothèse de convergences pour l'absence de chants est confirmée par l'analyse phylogénétique , la progressivité des pertes n'est cependant pas corroborée, et la polarisation des absences ou présences des chants n'est pas forcément celle prédite par le modèle Des réversions sont par contre documentées, ce qui n'avait jamais été envisagé auparavant L'évolution acoustique apparaît finalement fortement homoplasique chez les grillons Les analyses phylogénétiques montrent également que les facteurs tels que prédation structure de populations, , ne peuvent pas être caractérisés les uns par rapport aux autres par leur effet supposé sur la communication acoustique les hypothèses évolutives proposées à leur sujet ne sont pas exclusives et ne peuvent dans leur forme actuelle se prêter à une procédure de test Bien que la communication acoustique des grillons ait fait l'objet de nombreuses études, aucune hypothèse claire n'existe actuellement sur son origine et ses modalités d'évolution Des études conjointes en phylogénie et en biologie des populations seront ainsi nécessaires d'une part pour généraliser les résultats déjà obtenus sur les Phalangopsidae, et d'autre part pour proposer de nouvelles hypothèses sur la question.

INTRODUCTION

Acoustic communication plays a leading role in cricket biology In most species it is associated with mating Songs are emitted by males only (Fig 1), either to attract distant females (calling songs), to attract and keep the females at close range (courting songs) or to chase male intruders (aggressive songs) (CHOPARD, 1938, HUBER *et al*, 1989) Singing is achieved by means of a special forewing apparatus called the stridulum (Figs 2-8) This apparatus is complex, both regarding its structure and its operative mode (MICHELSEN & NOCKE, 1974, SISMONDO, 1979, KOCH *et al.*, 1988, BENNET-CLARK, 1989, DESUTTER-GRANDCOLAS, 1995a), and it is widely and exclusively distributed in crickets It is thus currently considered ancestral in this clade (ALEXANDER, 1962, 1967, OTTE, 1977, 1992, WALKER & MASAKI, 1989)

The question of how acoustic behavior has evolved in crickets has long been debated ALEXANDER (1962, 1967, 1987) postulated that originally cricket songs were similar to courting songs, emitted at close range The subsequent evolution of acoustic communication in crickets would have been achieved by the diversification of the emitted signals, which would have been driven by two factors the growing number of potentially interacting acoustically signaling species (each species being characterized by at least its calling song), and an increase in the number of functions for the signals ALEXANDER (*op. cit*) thus assumed that the calling song derived from the courting song, and the aggressive song from the calling song (see also BAILEY, 1991) "The only soft, close-proximity signals among modern crickets are courtship sounds, and it is likely that this reproductive context was the one in which the first cricket chirp was produced All the other signals are probably outgrowths of this fundamental situation" (ALEXANDER, 1987: 84)

The acknowledgment that not all crickets are able to sing (Figs 3, 6, 8) has led other authors to consider that singing may have been lost many times in crickets This evolution toward muteness has been hypothesized to follow several steps based upon the life habits of extant species (OTTE, 1977, 1990, 1992, WALKER & MASAKI, 1989, BAILEY, 1991) These steps, outlined in figure 9, include 1) Ancestrally, species sang and had three song types 2) In some circumstances, the calling song may have become facultative, singing and non-singing (satellite) males living in close proximity 3) The calling song was definitively lost, but courting and aggressive songs still existed 4) Species became mute, even though they still retained the stridulum 5) The stridulum was finally lost This loss may or may not have been followed by the loss of auditory organs (OTTE, 1990)

Source MNHN, Paris

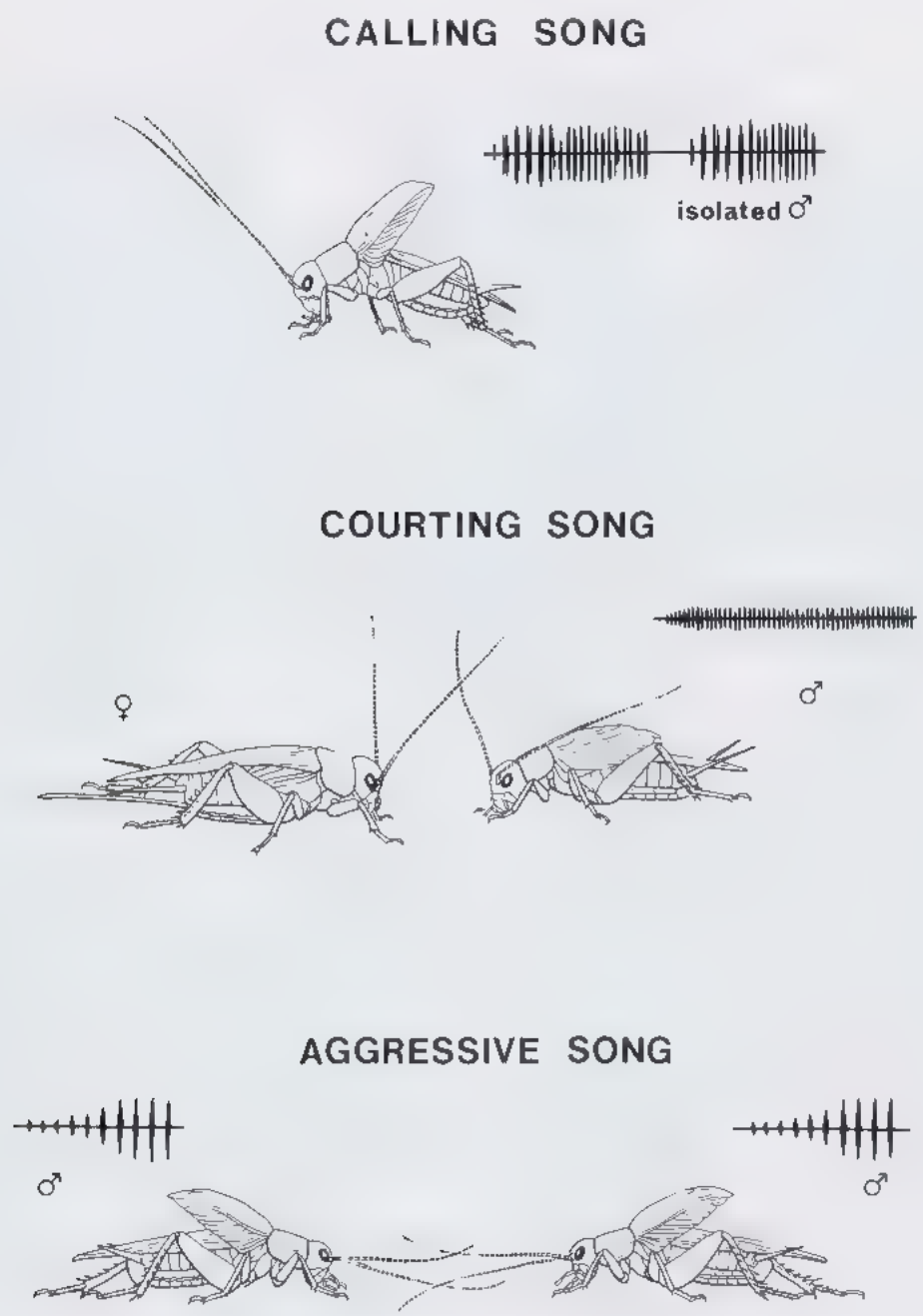


FIG 1 The three main songs emitted during mating by crickets (modified from LOHER & DAMBACH, 1989) Sonagrams of the songs in frames

Current hypotheses on the acoustic evolution in crickets thus assume that 1) songs have evolved progressively from the courtship song, 2) the ancestral stridulum and songs have been lost several times in different cricket lineages, and 3) both the loss of the stridulum and that of the songs have been achieved according to a definite and linear transformation series No reversal of this gradual song loss has ever been hypothesized In order to analyze the evolution of

Source MNHN, Paris

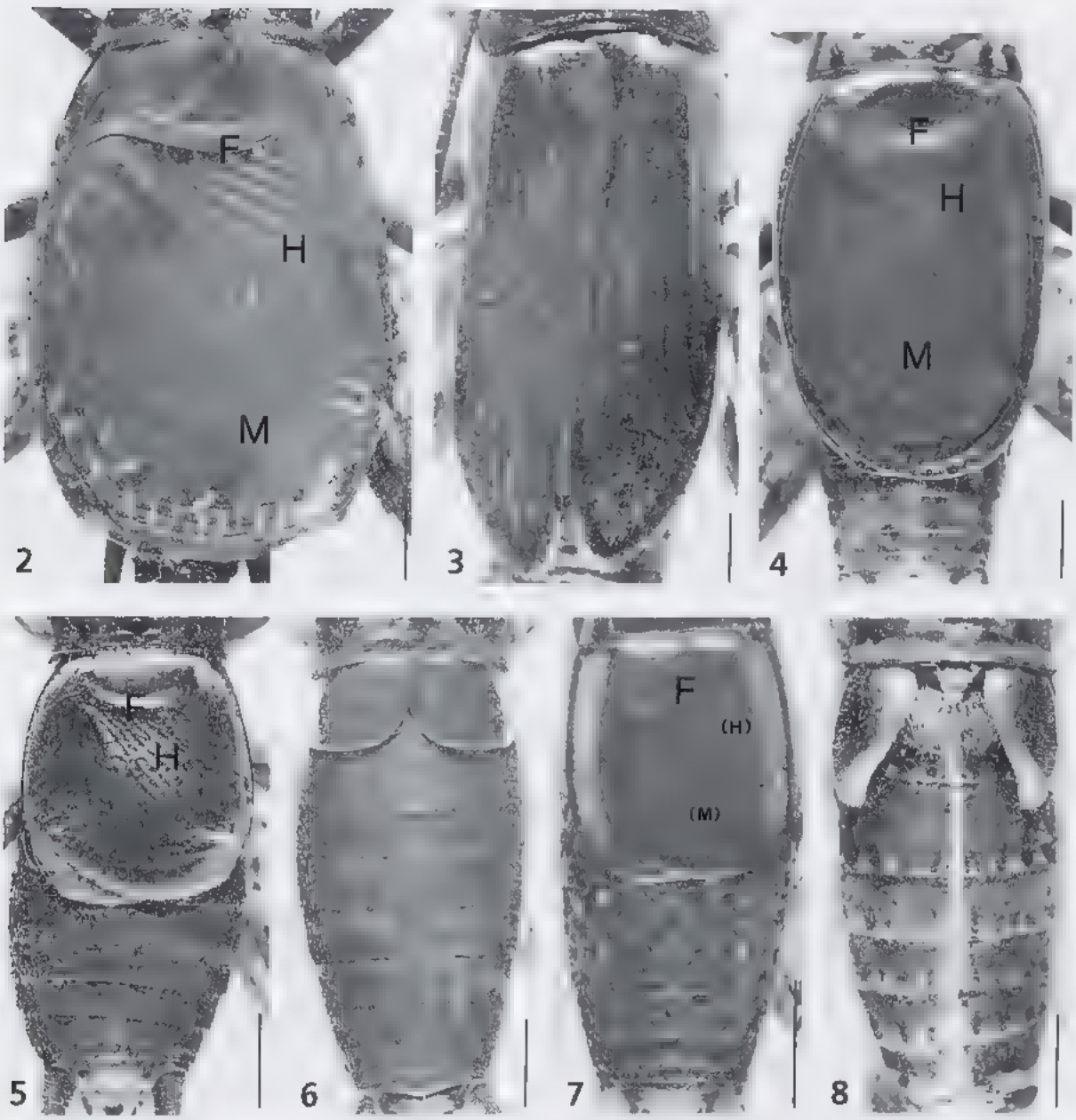
acoustic communication in crickets, it is necessary to consider separately the evolution of the stridulum and that of the acoustic repertoire Although these traits are obviously connected (in crickets, song *s.str.* is only emitted by the stridulum), there exists no obligatory correspondence between definite states of the stridulum and the extent of acoustic repertoires (OTTE, 1977, 1992)

Hypotheses of stridulum loss have been tested in a phylogenetic context (DESUTTER-GRANDCOLAS, 1997) Phylogenetic patterns support the hypothesis of a convergent loss of the stridulum They did not support, however, the progressive disappearance of the stridulum a functional stridulum could be lost in only one evolutionary step, without intermediary conditions A high level of homoplasy was also documented for diverse stridulum types, and phylogenetic patterns indicate that reversal could occur Finally the stridulum appeared evolutionarily labile

I will consider here the hypotheses about the evolution of the cricket songs I will not however analyze whether courting is the ancestral song type in crickets, as this would have to be tested at a higher phylogenetic level Supposing that the ancestral acoustic repertoire of true crickets comprises a calling, a courting and an aggressive songs, current assumptions on their acoustic evolution could be described by a definite sequence of song combinations (Fig 9) This sequence implies that the loss of the songs is ordered, the calling song disappearing first, followed by the courting and the aggressive songs Given this only three of the eight possible combinations of the three songs should exist (Fig 10) Again no reversal is hypothesized Here I will perform phylogenetic tests of these theoretical patterns and ask if song loss is the only possible evolutionary change in the acoustic evolution of crickets

Dealing with the patterns of acoustic evolution in crickets, one cannot help asking which factors may have influenced it Four factors have been hypothesized to have played a role in the evolutionary reduction of cricket acoustic repertoire (HUBER *et al*, 1989, OTTE, 1992). Is it possible first to characterize the potential influence of each factor, and second to test it using phylogeny? The first, and most strongly advocated factor is predation Both parasites and predators are supposed to be attracted by calling individuals, thereby influencing long range signals (CADE, 1975, BURK, 1982, THORNHILL & ALCOCK, 1983, BAILEY, 1991) For crickets, this means that the calling song could be affected, but not the courting or the aggressive songs, which are emitted at short range The second factor is the environment, in particular the environment's effect on the efficiency of acoustic signal transmission (RÖMER, 1993) Communication occurs between a sender(s) and a receiver(s) Efficient communication allows the receiver(s) to know who calls, what for and from where Physical problems in sound propagation in the natural environment may alter the information conveyed by acoustic signals, especially for pure-tone signals such as cricket calls (MICHELSEN & NOCKE, 1974, RÖMER & LEWALD, 1992 *in* RÖMER, 1993) Acoustic signals emitted simultaneously can also mask each other (RÖMER, 1993) Finally some environments have been supposed unfavorable for acoustic communication because of their physical properties or because of their noisiness (for example caves or shores, respectively OTTE, 1992) Environmental constraints are thus more likely to interfere with long range signals (RÖMER, *op cit*) than with short range signaling Population structure and habitat have been hypothesized to influence song loss via sedentariness (WALKER, 1974) or confinement (BOAKE, 1984a, b), respectively (see also ALEXANDER, 1962) The idea is that individuals that stay together can find each other by chance without any special attractant

Source MNHN Paris



FIGS 2-8 Diversity of tegminal structures in phalangopsid crickets. **2**: *Noctivox sanchezi* (Amphiacustae) with a normally developed non corneous stridulum. **3**: *Phaeophilacris* sp. with tegmina modified for communication through air puffs (DAMBACH & [illegible] 1978). **4**: *Luzarida guyana* (*Luzarida* group), with a normally developed stridulum but a corneous right tegmen. **5**: *Paragryllodes* sp. with a reduced though functional stridulum. **6**: *Cantrallia huasteca* (Amphiacustae) with non overlapping tegmina and no functional stridulum. **7**: *Luzaridella clara* (*Luzarida* group) with an incomplete stridulum and a corneous right tegmen. **8**: *Ludmanacris multispinosa* with deeply modified tegmina probably showing glandular structures ([illegible]-GRANDE 1994b). Stridulum F: file, H: harp, M: mirror. Scales: 2 mm.

Source: MNHN, Paris

CURRENT MODEL FOR ACOUSTIC EVOLUTION IN CRICKETS

1/ Ancestral condition: 3 song types *(calling, courting, aggressive song)*

2/ Multiple, independent losses

3/ Several definite steps toward muteness

Calling song facultative (satellite males) (call.* court. aggr.)
Calling song lost (- court. aggr.)
Other songs lost (- - -)
Stridulum (+/-auditory organs) lost

4/ No reversal hypothesized

FIG. 9 — Current model on the evolution of acoustic communication in crickets (references in the text).

SONG COMBINATIONS PREDICTED BY THE TESTED MODEL

1/	Call.	Court.	Aggr.
2/	-	Court.	Aggr.
3/	-	-	-

SONG COMBINATIONS NOT PREDICTED BY THE TESTED MODEL

4/	Call	Court	-
5/	Call	-	Aggr
6/	Call	-	-
7/	-	Court.	-
8/	-	-	Aggr

FIG. 10. — List of song combinations that could exist in crickets. Names of songs: Call: calling song, Court: courting song, Aggr: aggressive song (references in the text).

signal. Here again the long-range signal would be lost. The fourth factor that has been hypothesized to influence song evolution is the evolutionary shift toward another communication mode. Chemical (OTTE, 1977, 1992) and visual (TOMS, 1986, BAILEY, 1991) shifts have been proposed as replacement communication systems. Vibrational communication has also been recorded in crickets (LOHER & DAMBACH, 1989), however there is currently no suggestion that it replaced acoustics. Chemical and visual signals are efficient at both long and short range, visual

Source: MNHN, Paris

FACTORS CURRENTLY HYPOTHESIZED TO BE RESPONSIBLE FOR ACOUSTIC EVOLUTION OF CRICKETS, AND THE SONG REPERTOIRE THEY IMPLY

1/ Predation: (Call Court Aggr) » (- Court Aggr)

2/ Inefficiency of acoustic communication (Call Court. Aggr.) » (- Court. Aggr)

3/ Population structure / Habitat (Call Court. Aggr) » (- Court Aggr)

4/ Shift toward another communication mode

A/ At long range only (Call Court Aggr) » (- Court Aggr)
B/ At long range, and at short range between M/F and M/M (Call Court Aggr.) » (- - -)
C/ At long range, and at short range between M/M (Call Court. Aggr) » (- Court. -)
D/ At long range, and at short range between M/F (Call Court Aggr) » (- - Aggr)
E/ At short range between M/F (Call Court Aggr) » (Call - Aggr)
F/ At short range between M/M (Call. Court Aggr.) » (Call Court -)
G/ At short range between M/F and M/M (Call Court. Aggr) » (Call. - -)

FIG 11 — Current hypotheses on the factors that could have influenced the acoustic evolution of crickets (references in the text).

cues being efficient only in daylight At long range, these signals would replace the calling song At short range, they could play a role in interactions between male and female and replace the courting song, or between males only and replace the aggressive song, or in both kinds of interactions, replacing both the courting and aggressive songs Operating over both long and short ranges, these signals could potentially replace all types of songs

Figure 11 shows the different song combinations that would result from the influence of each factor on the evolution of acoustic communication in crickets It is clear that these factors are not mutually exclusive, and that a given sequence of songs is predicted by more than one factor For example, the loss of the calling song is expected from the influence of predation, inefficiency of acoustic communication, population structure, habitat type or a shift toward another long range communication mode This overlap precludes a test of the influence of these factors Some song combinations, however, appear specific of one factor

I present here a phylogenetic test of current hypotheses on the modalities of the acoustic evolution in crickets, and on the factors that could have influenced it For this I will confront the theoretical patterns these hypotheses imply with the results of my phylogenetic analyses on two monophyletic cricket clades, the Amphiacustae and the *Luzarida* group (Grylloidea, Phalangopsidae) In each clade the optimization of song types onto the phylogeny allows me to derive evolutionary scenarios on the acoustic evolution of the clade These scenarios may or may not fit the theoretical patterns and may or may not corroborate the hypotheses under study (CODDINGTON, 1990, CARPENTER, 1989, BROOKS & MCLENNAN, 1991, MCLENNAN, 1991, GRANDCOLAS *et al* , 1994) For practical reasons, the hypotheses on the factors will be analyzed using the Amphiacustae clade only

Source MNHN Paris

MATERIAL AND METHODS

Two monophyletic groups of phalangopsid crickets (Grylloidea, Phalangopsidae) were used in this study: the Amphiacustae (DESUTTER-GRANDCOLAS, 1993a, 1994a) and the *Luzarida* group (DESUTTER-GRANDCOLAS, 1993b). Their phylogeny has been previously analyzed with cladistics, using Wagner parsimony and the option implicit enumeration of the Hennig86 program (FARRIS, 1988). Data matrices were built with unweighted morphological and anatomical characters; multistate characters were coded as non additive. No song characters were then included in the matrices because of the lack of evident primary homologies (DE PINNA, 1991; GRANDCOLAS *et al.*, 1994).

For the present paper, song data were collected from the literature and from my own personal observations in the field. They were treated as attributes (MICKEVICH & WELLER, 1989) and optimized on the cladograms using Wagner parsimony. Each song was treated as one attribute, with two possible states (present/absent). Three attributes were considered: calling song (Call), courting song (Court) and aggressive song (Aggr).

The Amphiacustae (Figs 12, 14) comprise nine genera distributed in Central America and the West Indies. Cladistic analyses of morpho-anatomical characters resulted in one phylogenetic tree (CI = 0.92, RI = 0.95, 28 steps) (DESUTTER-GRANDCOLAS, 1993a, 1994a). Two monophyletic species groups exist in the genus *Mayagryllus*: one group (*Mayagryllus* 1) presents no tegmina, that is no acoustic apparatus, the other (*Mayagryllus* 2) includes two apterous species (no stridulum) and one species with reduced, not corneous tegmina and a functional stridulum. Songs have been described by ALEXANDER & OTTE (1967) for *Amphiacusta* and BOAKE (1983, 1984a, b) for *Nemoricantor*. I have observed *Noctivox* and *Cantrallia* in their natural habitat. *Arachnopsita*, *Leptopedetes* and *Mayagryllus p.p.* have tegminal conditions that do not allow them to sing (DESUTTER-GRANDCOLAS, 1993a, 1996). No data exist on *Longuripes*, *Prolonguripes* and *Mayagryllus p.p.*

The *Luzarida* group (Figs 13-15) comprises nine genera distributed in the northern half of South America, east of the Andes. Cladistic analyses of morpho-anatomical characters resulted in one, incompletely resolved tree (CI = 0.80, RI = 0.86, 20 steps) (DESUTTER-GRANDCOLAS, 1993b). All available data on the singing behavior of the *Luzarida* group taxa (except *Palpigera*, the song of which is unknown) result from my own personal observations in the field.

RESULTS

The Amphiacustae

Song evolution. The states of the attributes are at least partly documented in 7 of the 9 genera of the Amphiacustae, and a complete series of attributes states is available for 5 of them (plus *Mayagryllus p.p.*). Acoustic communication has been completely described for 2 taxa (*Amphiacusta*, *Nemoricantor*), it is absent in 3 others (*Cantrallia*, *Leptopedetes*, *Arachnopsita*), plus *Mayagryllus p.p.* Mapping song attributes onto the cladogram (Fig. 14) shows that the three song types are not obligatorily present in any singing taxa (although they could be in *Noctivox*). *Amphiacusta* has no aggressive song, while it has a calling and a courting song. *Nemoricantor* on the contrary has only a courting and an aggressive song, but no calling song. The combinations of attributes states found in the Amphiacustae are (Call, Court, -), (Call, Court, Aggr), (Call, Court, ?) and (-, -, -). All these combinations are predicted by the model depicted in Figure 9, except for (Call, Court, -). Also their distribution on the phylogeny of the Amphiacustae does not support the hypothesis of a linear transformation toward the loss of acoustic communication.

The scenarios derived for each attribute are as follows.

Calling song (Fig. 16). Three equally parsimonious scenarios exist, with two steps each.

A) Calling song is ancestral, it is lost twice independently in *Cantrallia* and in the clade [*Leptopedetes* - *Mayagryllus*].

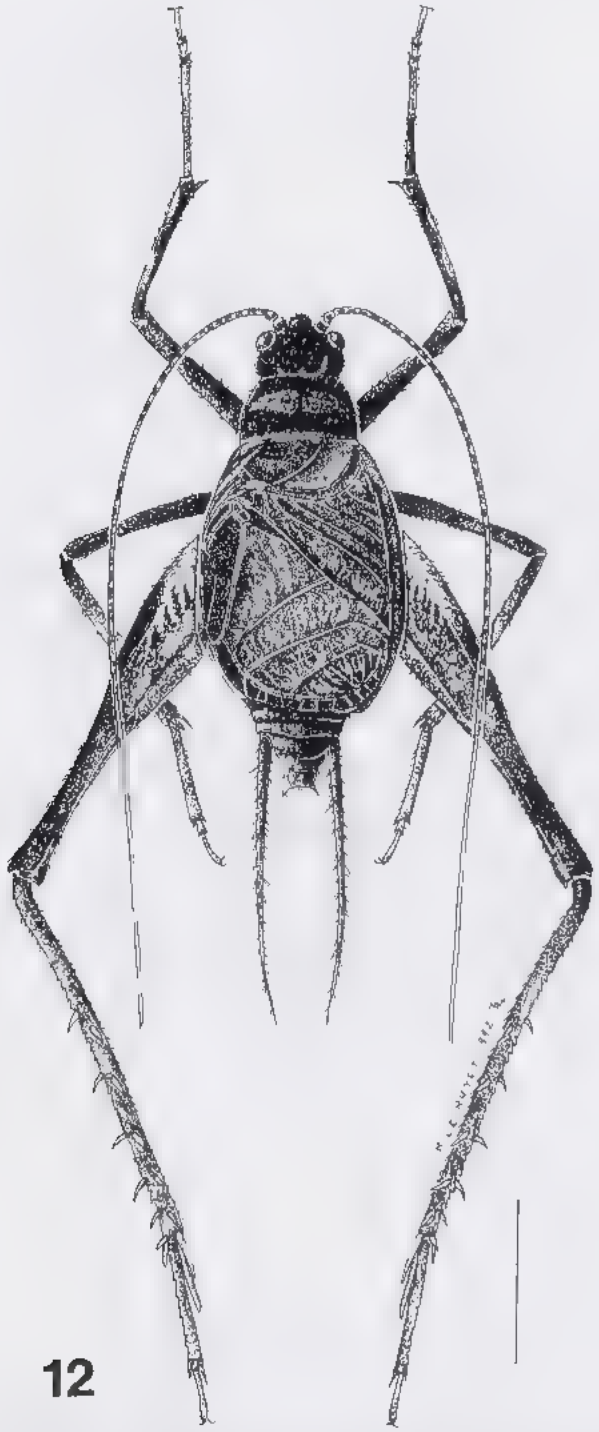
B) Absence of calling song is ancestral, a calling song appears once in [*Amphiacusta* (*Noctivox* - *Cantrallia*)], and one subsequent reversal to ancestral condition occurs in *Cantrallia*.

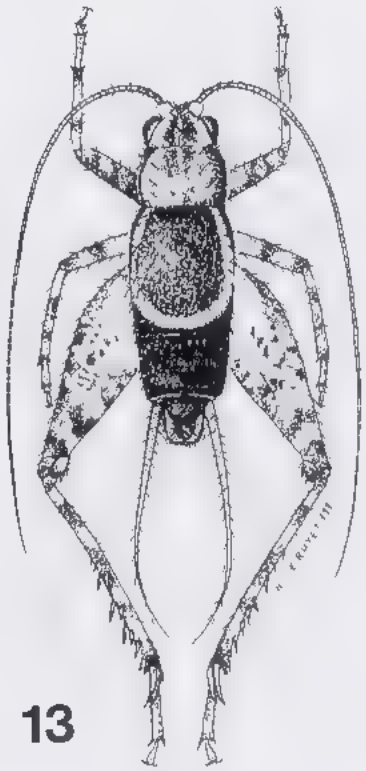
C) Absence of calling song is ancestral, two independent appearances of a calling song occur in *Amphiacusta* and *Noctivox*.

Courting song (Fig. 17). Four equally parsimonious scenarios exist, with 3 steps each.

A) Courting song is ancestral, three independent losses of courting song occur in *Cantrallia*, *Leptopedetes* and in the subgroup [*Arachnopsita* - *Mayagryllus*].

Source: MNHN, Paris





FIGS 12-13 — **12:** *Noctivox sanchezi* Desutter-Grandcolas, 1993a (Amphiacustae), scale 5 mm. Note the well-developed stridulum. **13.** *Ochraperites ottei* Desutter-Grandcolas 1993b (*Luzarida* group), scale 1 mm. Males in dorsal view, modified from DESUTTER-GRANDCOLAS, 1993a, 1993b. Note the consistency of tegmina and the type of stridulum (right tegmen with a stridulatory file only).

B) Courting song is ancestral, the courting song is lost twice independently in *Cantrallia* and in the clade [*Leptopedetes* - *Mayagryllus*], and one subsequent reversal occurs in *Nemoricantor*.
C) Absence of courting song is ancestral, a courting song appears twice independently in

Source: MNHN, Paris

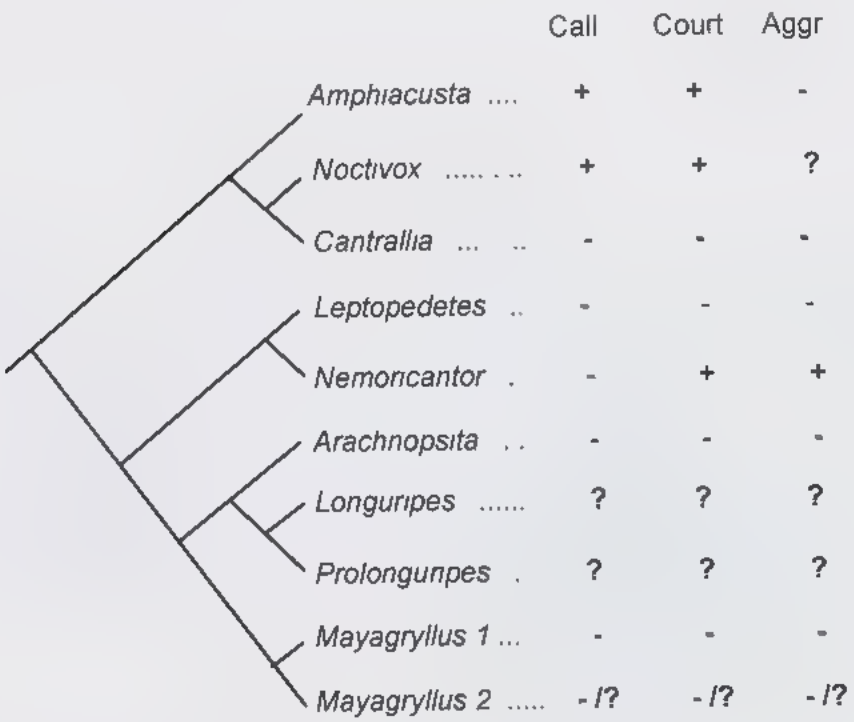


FIG 14 — Phylogeny and song attributes in the Amphiacustae (Grylloidea, Phalangopsidae) Symbols for attributes + presence, - absence, ? state unknown

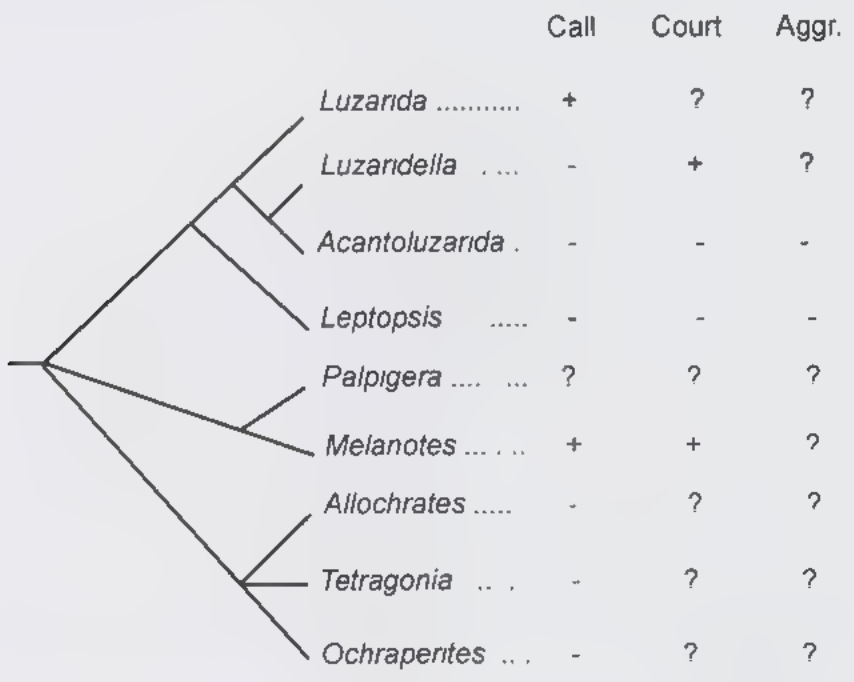


FIG 15 — Phylogeny and song attributes in the *Luzarida* group (Grylloidea, Phalangopsidae) Symbols for attributes + presence, - absence, ?' state unknown

Source MNHN Paris

Nemoricantor and in the clade [*Amphiacusta* (*Noctivox* - *Cantrallia*)], and one subsequent reversal to ancestral state occurs in *Cantrallia*

D) Absence of courting song is ancestral, three independent appearances of a courting song occur in *Amphiacusta*, *Noctivox* and *Nemoricantor*

Aggressive song (Fig. 18) Only one most parsimonious scenario has been found (1 step) It implies an ancestral absence of the aggressive song and its subsequent appearance in *Nemoricantor*.

A combined analysis of all three attributes shows that 12 equally parsimonious scenarios (6 steps) could explain the present distribution of song types in the Amphiacustae (Fig 19) The ancestral repertoire is ambiguous it may comprise a courting or a calling song, both songs or neither; the aggressive song is ancestrally absent in all 12 cases

All the scenarios show convergent modifications of the calling song, the courting song or both the songs appear or disappear, according to the ancestral condition, in two or more taxa For example, when the calling song is ancestrally absent, the scenarios always imply subsequent appearances of the calling song (Figs 19C - F, I - L), a similar situation occurs for the courting song (Figs 19G - L) Conversely, when the calling song (or the courting song) is ancestral in Amphiacustae, several convergent losses occur

Factors of song evolution. Comparison with the theoretical song combinations (Figs 10-11) shows that only three of them exist in the Amphiacustae (- Court Aggr) in *Nemoricantor*, (- - -) in *Cantrallia*, *Leptopedetes*, *Arachnopsita* and *Mayagryllus p.p.*, and (Call Court -) in *Amphiacusta* These could support a potential effect of the following factors predation, inefficient acoustic communication, population structure, habitat, evolution toward a pheromonal communication between males and females both at long and close range, and evolution toward a pheromonal communication between males at close range. One should remark however that these factors have always been supposed to have interfered with an ancestral song combination comprising all three song (Call Court Aggr) Such an ancestral song combination is however not attested for the Amphiacustae, as the Amphiacustae ancestrally lack an aggressive song This means that none of the evolutionary sequence hypothesized to test the influence of currently invoked factors is found in this group, and that no current hypothesis can account for present data on this clade

The Luzarida *group*

Song attributes are not as well known in the *Luzarida* group as in the Amphiacustae, especially for the courting and the aggressive songs (Fig 15) these attributes are known in four and two taxa respectively, two of them being deprived of a stridulum A complete description of the attributes is thus available only for those non acoustic taxa (combination - - -) Other incomplete combinations are (Call Court ?), (- Court ?), (Call ? ?) and (- ? ?) These combinations are not incompatible with the tested model of the evolution of acoustic communication in crickets According to available data, scenarios could be derived only for the calling and the courting songs

Calling song (Fig. 20) Only one most parsimonious scenario (2 steps) exists It implies an ancestral absence of the calling song, and two subsequent, independent appearances in *Luzarida* and in *Melanotes*.

Source MNHN Paris

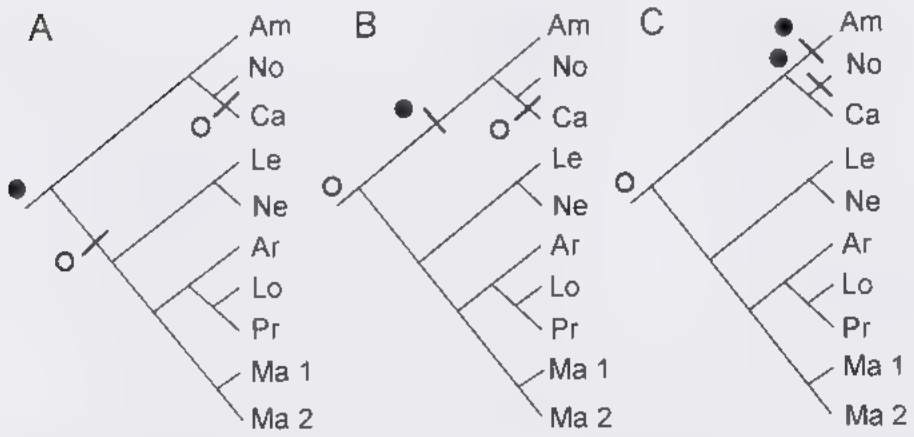


FIG 16 Equally parsimonious scenarios for the evolution of the calling song in the Amphiacustae Symbols black circle presence, empty circle absence, thick line evolutionary change Names of taxa Am *Amphiacusta*, Ar *Arachnopsita*, Ca *Cantrallia*, Le *Leptopedetes*, Lo *Longuripes*, Ma 1, 2 *Mayagryllus (1, 2)*, Ne *Nemoricantor*, No *Noctivox*, Pr *Prolonguripes*

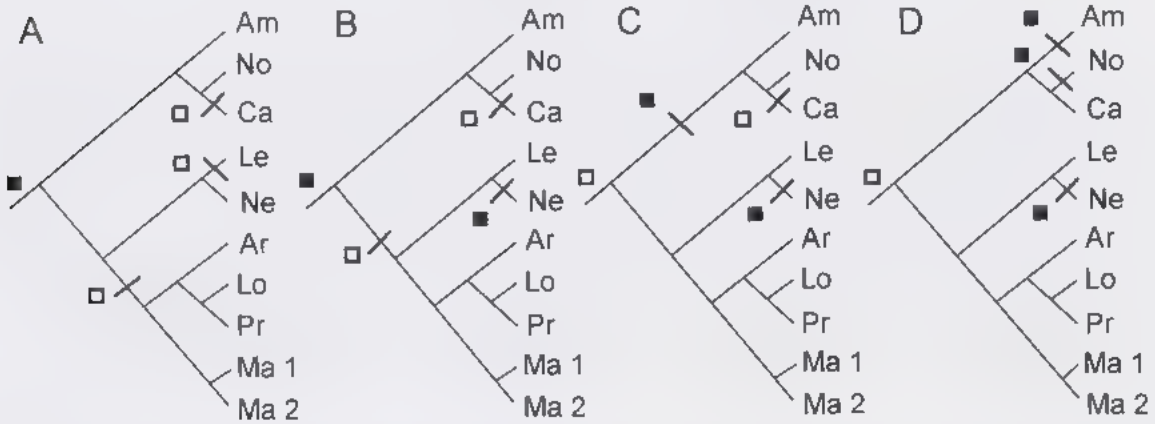


FIG 17 — Equally parsimonious scenarios for the evolution of the courting song in the Amphiacustae Symbols black square presence, empty square absence, thick line evolutionary change Names of taxa as in figure 12

Courting song (Fig. 21) Three equally parsimonious scenarios (2 steps) are possible

A) Courting song is ancestral, it disappears twice independently in *Leptopsis* and in *Acantoluzarida*

B) Courting song is ancestral, it is lost in the clade [*Luzarida* - *Leptopsis*] A subsequent reversal occurs in *Luzarida*

C) Absence of courting song is ancestral, a courting song appears twice independently in *Luzaridella* and in *Melanotes*.

The combined analysis of the calling and courting songs (Fig 22) shows that 3 equally parsimonious scenarios exist for the acoustic evolution of the *Luzarida* group They all have 4

Source MNHN Paris

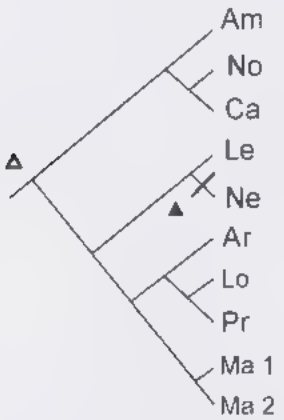


FIG 18 — Parsimonious scenario for the evolution of the aggressive song in the Amphiacustae Symbols black triangle presence, empty triangle absence, thick line evolutionary change Names of taxa as in figure 16

steps and imply convergent changes of the calling song and, for two of them, of the courting song too The ancestral condition is absence of calling song, and presence or absence of courting song It should be noted that in all these scenarios the ancestrally absent calling song reappeared twice independently, which does not corroborate the tested model

DISCUSSION

What is the pattern of the evolution of acoustic communication in crickets?

Even if acoustic behavior is still incompletely known in the Amphiacustae and in the *Luzarida* group, the phylogenetic analyses of the available songs partly invalidate current proposals on the acoustic evolution of crickets As already indicated above, the only hypothesis which cannot be tested with these data is whether the courting song is the ancestral song for crickets (ALEXANDER, 1967, BAILEY, 1991) However a courting song exists in all the taxa which emit acoustic signals, while calling and aggressive songs may be absent

I will consider the following questions in turn are song losses documented? Are the observed song combinations similar to those predicted by the model? Is the hypothesis of a linear (regressive) transformation of acoustic repertoire attested by the phylogenetic patterns?

Songs are lacking in several taxa in the studied clades This lack may concern the whole three songs (mute taxa) or only one of them The missing song is then either the calling song (*Nemoricantor* in the Amphiacustae, *Luzaridella* in the *Luzarida* group) or the aggressive song (*Amphiacusta* in the Amphiacustae) Both the absence of the calling song and the taxa muteness could support the model of a regressive evolution of cricket acoustic repertoire (Fig 9) The absence of the aggressive song is however not consistent with it Also the polarization of song absence according to phylogenetic patterns suggests that a song absence in a taxon does not necessarily mean that the song has been lost in that taxon Song lack may be ancestral to a whole clade This means that song lack can be apomorphic or plesiomorphic, and this also is not consistent with the model

Source MNHN Paris

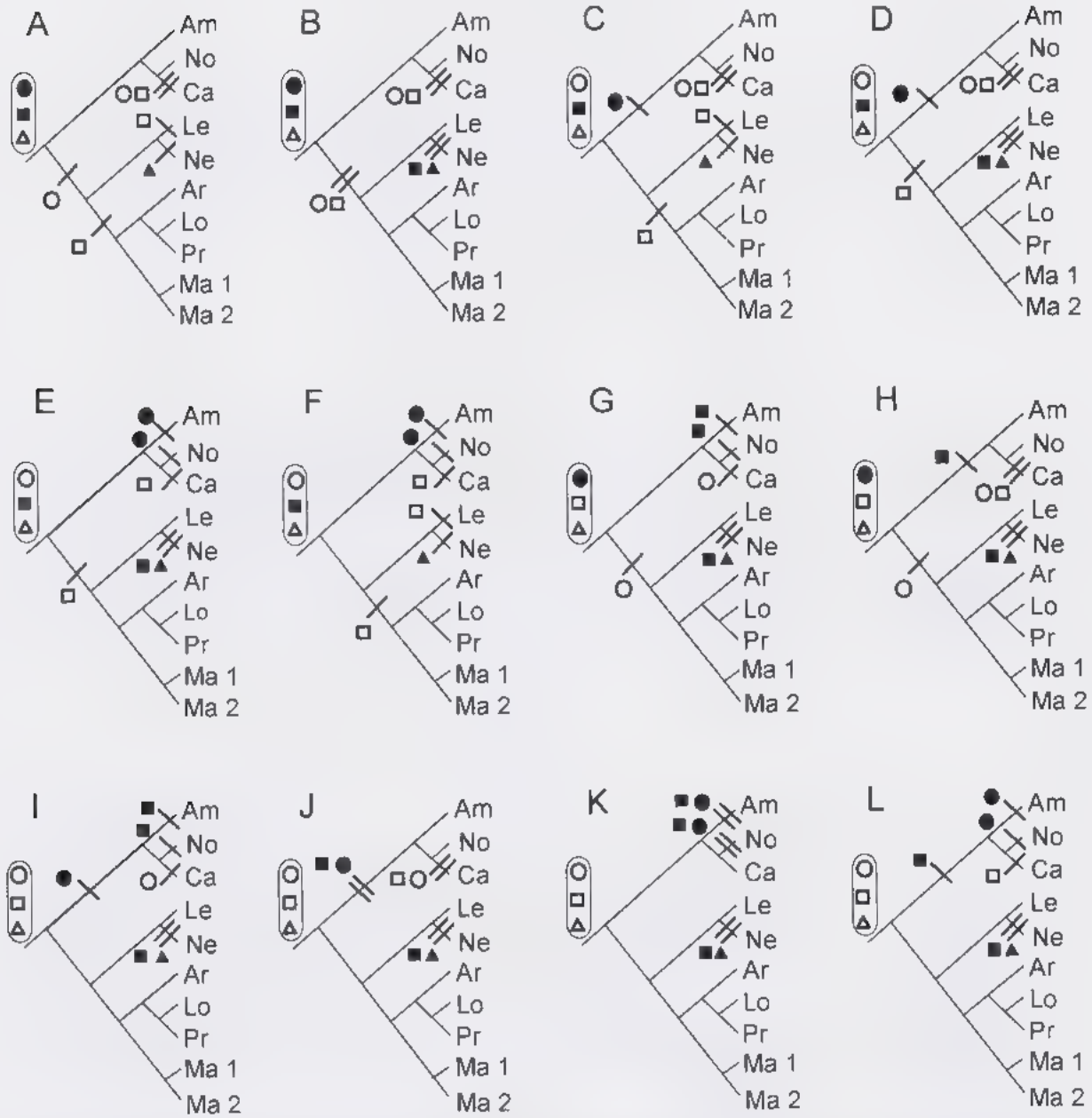


FIG. 19 Equally parsimonious scenarios for the evolution of singing ability in the Amphiacustae. Symbols as in figures 16-18, ancestral states of attributes indicated in a frame. Names of taxa as in figure 16.

The song combinations assumed by the model shown in Figure 10 include (Call Court Aggr.) as the ancestral condition, with (- Court Aggr.) and (- - -) as derived conditions. The last two combinations have been documented here. As mentioned above, however, the combination (Call Court Aggr.) does not represent the ancestral condition in the studied groups: the Amphiacustae ancestrally lack an aggressive song, while the *Luzarida* group is

Source: MNHN, Paris

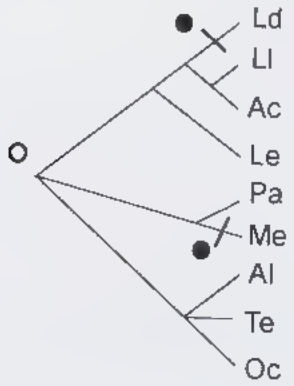


FIG 20 Parsimonious scenario for the evolution of the calling song in the *Luzarida* group Symbols black circle presence, empty circle absence, thick line evolutionary change Names of the taxa Ac *Acantoluzarida*, Al *Allochrates* Le *Leptopsis*, Ld *Luzarida*, Ll *Luzaridella*, Me *Melanotes*, Oc *Ochraperites*, Pa *Palpigera*, Te *Tetragoma*

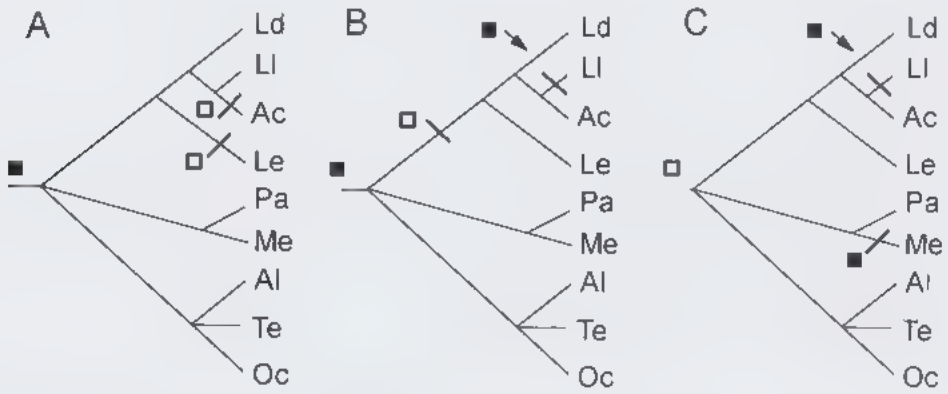


FIG 21 — Equally parsimonious scenarios for the evolution of the courting song in the *Luzarida* group Symbols black square presence, empty square absence, thick line evolutionary change Names of taxa as in figure 20

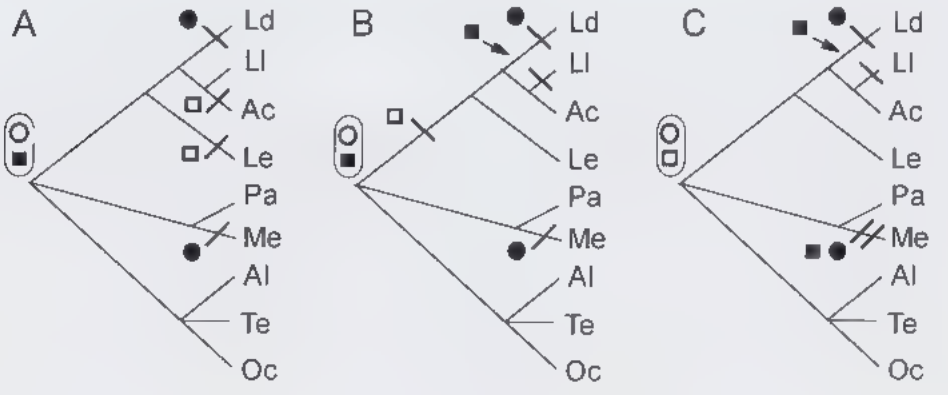


FIG 22 — Equally parsimonious scenarios for the evolution of the singing ability in the *Luzarida* group Symbols as in figures 20-21, ancestral states of attributes indicated in a frame Names of taxa as in figure 20

Source MNHN Paris

ancestrally deprived of a calling song. If the combination (Call, Court, Aggr.) was to occur in these clades, owing to additional data for presently unstudied taxa, it would consequently constitute a derived condition. Moreover, the combination (Call, Court, -), which characterizes *Amphiacusta*, is not predicted by the model. Again the current model on the acoustic evolution of crickets is only partly supported and is unable to explain the observed situation. Similarly, none of the phylogenetic patterns presented here is congruent with the theoretical patterns of figure 9, which means that the hypothesis of a linear loss of the songs is not supported by either the Amphiacustae or the *Luzarida* group case studies.

The fact that current hypotheses on the acoustic evolution of crickets are not supported by the phylogenetic analyses of the presence/absence of the songs, means that this evolution cannot be summarized as mere multiple, progressive losses of songs. The phylogenetic analyses show that neither the song combinations nor the polarization of character changes are only those predicted by the model: other possibilities are documented, while some of those predicted by the model are not supported. Phylogenetic patterns also suggest additional aspects of song evolution in crickets that have never been expected before. First, reversals may occur. For example a song which was ancestrally lacking in a cricket clade could reappear in a subclade. Such is the case for the calling song in *Luzarida* and *Melanotes* in the *Luzarida* group. Also convergent changes are common in cricket clades, and there is no obligatory series between the possible song combinations. Similar conclusions were drawn from phylogenetic analyses of stridulum evolution (DESUTTER-GRANDCOLAS, 1996).

The complexity of the phylogenetic patterns that described the evolution of song and stridulum in the studied cricket clades is not a unique phenomenon. Many authors have re-examined evolutionary hypotheses in a phylogenetic framework and documented complex phylogenetic patterns, among which reversals are far from being unusual (ANDERSEN, 1979, 1994, CODDINGTON, 1988, CARPENTER, 1989, WANNTORP *et al.*, 1990, BROOKS & MCLENNAN, 1991, PACKER, 1991, SIDDALL *et al.*, 1993, DESUTTER-GRANDCOLAS, 1993a, 1994a, ANDERSEN & WEIR, 1994, GRANDCOLAS, 1996, many contributors, this volume). One consequence of these results however is that the evolution of acoustic communication in crickets may have been much more complicated than previously thought, at least in some cricket clades. The previous model constructed to explain the evolution of acoustic communication in crickets *s.l.* hypothesized quite simple transformation series. These series were in turn documented in relatively homogeneous groups (mostly gryllid taxa), which populations could be easily studied. When a wide diversity of tegminal structures and communication signals is involved, as in Phalangopsidae for example (Figs 2-8), this model becomes inefficient. GRANDCOLAS *et al.* (1997, this volume) denounced the sampling bias that can be generated in phylogenetic reconstructions by the properties of the clades under study: a clade that presents a wide diversity of features has experienced a larger number of evolutionary events than a clade which is relatively homogeneous for the same features. The study of a diverse clade may consequently lead to overestimate the frequency of evolutionary changes and events. On the reverse, studies of poorly diverse clades may conclude to low frequencies of evolutionary transformations. These biases do not invalidate the results obtain in each case. On the contrary a general theory on the acoustic evolution of crickets will have to explain the complicated cases documented in the Phalangopsidae, as well as the more simple ones that could be found in other cricket groups.

Source: MNHN, Paris

Which factors may have influenced the acoustic evolution of crickets?

Answers to this question have always been based on the assumption that acoustic signals only evolve by song and stridulum loss in crickets (ALEXANDER, 1962, 1967, OTTE, 1977, 1992, WALKER & MASAKI, 1989, BAILEY, 1991) A similar approach was adopted by studies of population biology dealing with song abilities and mating success (THORNHILL & ALCOCK, 1983, HUBER *et al.*, 1989, BAILEY, 1991) The phylogenetic analyses presented here clearly demonstrate that such is not the case on the contrary, the acoustic evolution of crickets involves a high level of homoplasy The hypotheses formulated up to now to explain the acoustic evolution of crickets have thus always been biased from the start, because an unwarranted hypothesis (a supposed evolutionary tendency to acoustic loss) was considered attested As such, these hypotheses are unable to test whether loss actually occurred, or whether other evolutionary changes may have existed One consequence is that no sound hypothesis exists now on the factors that could have influenced the acoustic evolution of crickets Another problem, as already mentioned previously, is that the hypotheses that have been proposed up to now are not mutually exclusive Thus the combination (- Court Aggr) could be used as evidence for the influence of predation, inefficiency of acoustic communication, population structure and habitat Ultimately this means that the hypotheses that have been proposed up to now on the subject could be conclusively tested in a population perspective but not in a historical perspective, although they are supposed to concern evolutionary processes *sensu lato*

What arguments have been used to support these hypotheses of acoustic evolution of crickets? And have they already been analyzed in a phylogenetic framework? Although cricket predation *s.str.* by many vertebrates and invertebrates has been recorded (WALKER & MASAKI, 1989), its actual pressure has never been measured Predation by bats in particular has been assumed to be heavy Some cricket species do show a high acoustic sensibility to ultrasound stimuli, which has been demonstrated to induce negative phonotaxis in flight patterns (SALES & PYE, 1974, HUBER *et al.*, 1989, HOY, 1991) Many mute taxa live however in habitats that are not accessible to bats, such as leaf litter, tree hollows, burrows, etc , and still more acoustic or non-acoustic species do not fly The effect of parasites on the other hand, especially that of tachinid flies, has been documented in populations of a few cricket species In these infested populations, some silent males, called satellites, stay near calling males and try to intercept the females attracted by the songs of the calling males (CADE, 1975) It has been suggested that this behavior could be an adaptation to avoid parasitoid infestation and constitute an alternative strategy for mating (THORNHILL & ALCOCK, 1983, BAILEY, 1991) ADAMO *et al.* (1995) show however in *Gryllus integer*, *G. bimaculatus* and *G. rubens* that infestation enhances the tendency of male crickets to mate, at least until tissue damage by the parasite is too high Also ZUK *et al* (1995) demonstrate that in a polymorphic population of *Teleogryllus oceanicus* silent males were either parasitized, or able to switch to calling behavior depending on population density The effect of parasites on calling behavior is thus manifold in cricket populations and depends on the conditions in which the populations live Its effect on the evolutionary change of acoustic behavior is then hard to predict for the moment until changes may have been actually fixed in taxa (SCHULTZ *et al.*, 1996) A phylogenetic test of predation pressure could be achieved by optimizing escape and acoustic behaviors displayed by the taxa, additional field work is then necessary to characterize such behaviors

Source MNHN, Paris

The role of the habitat in the acoustic evolution of crickets has been tested using phylogeny in the Amphiacustae (DESUTTER-GRANDCOLAS, 1995b) In this study, the phylogenetic patterns suggested that the habitat alone cannot have been a sufficient factor to drive the acoustic evolution of crickets For example taxa living in caves either are wingless, or have a complete, functional stridulum Similarly in one given habitat, several acoustic behaviors can be found (DESUTTER-GRANDCOLAS, *op. cit*) Population structure could be a more promising factor in this matter, but unfortunately field data are extremely sparse Only one taxon, *Nemoricantor maya* (Amphiacustae), has been studied in natural and laboratory conditions it is gregarious, living in hollow trees, and has no calling song (BOAKE, 1984a, 1984b) Lack of comparative data impedes attempts to determine the role of habitat and population structure on song evolution Here again combined analysis of phylogeny on one hand, and habitat and population structure on the other should permit a test of the gregariousness hypothesis

Finally, male crickets may have glands in many parts of their body Metanotal glands are better known, but others exist on the hindtibiae, the wings, the tergites, the base of some sclerites in male genitalia, etc (OTTE, 1992, DESUTTER-GRANDCOLAS, 1995b) The only phylogenetic analyses to date of glandular evolution in male crickets (Amphiacustae DESUTTER-GRANDCOLAS, 1995b) uncovered no shift from acoustic to chemical communication systems, except for the absence of metanotal gland in wingless taxa (probably for lack of protective structure for the glands)

Up to now, most studies on the acoustic evolution of crickets have combined assumptions on the patterns and assumptions on the processes, deriving the one from the other Phylogenetic analyses confront a phylogenetic pattern, built with as few hypotheses as possible, with independently constructed hypotheses on the evolutionary processes (GRANDCOLAS *et al*, 1994) They actually test the hypothesized processes with the phylogenetic patterns, the independence of the two sets of assumptions giving this method its power (GRANDCOLAS *et al*, 1997, this volume) Phylogenetic analyses have been applied here for the first time to the acoustic evolution of crickets These analyses have demonstrated that current hypotheses on the matter are largely insufficient and biased Instead they suggest far less simple scenarios for this evolution with high homoplasy They also clearly demonstrate that no sound hypothesis exists now on the factors that could have influenced cricket acoustic evolution In fact more phylogenies and more population studies are needed to build new hypotheses.

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Source: MNHN, Paris

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Source: MNHN, Paris

Defense Strategies in Scale Insects: Phylogenetic Inference and Evolutionary Scenarios (Hemiptera, Coccoidea)

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ABSTRACT

The sedentary plant-parasitic habit of scale insects increases their vulnerability to natural enemies and to adverse environmental factors. They have evolved a range of defense strategies which improve their chances of survival and reproductive success. These strategies are reviewed here. They include 1. construction of protective structures from secretions and/or excretions produced by the scale insects, 2. behavioral adaptations to exploit host-plant afforded protection, 3. modifications of their life-cycle in response to environmental factors and 4. modifications of the female body to provide protection for their progeny. The structure and formation of these protective structures were examined using SEM and by experimentation. A cladistic phylogenetic analysis of scale insects using 54 morphological characters and 25 genera resulted in two most parsimonious trees. This phylogeny was then used as a reference to provide hypotheses regarding the ancestral state and the subsequent evolution of habitat choice and the type of protective structure, especially in the nymphal stages. Phylogenetic analysis suggests that the ancestral habitat attribute (feeding site) is equivocal and currently we can not choose between the aerial (bark) or underground (roots) habitats. The subsequent adaptation led to colonization of branches, leaves and grasses. Most successful groups are adapted to the aerial part of woody host plants, particularly on the branches, which are the most exploited feeding sites by scale insect populations today. The ancestral type of protection is inferred to have been amorphous secretions. Subsequently, different clades probably independently developed protective tests with different combinations of secretions and anal fluid components, with a trend to find a compromise between energy costs and efficiency.

RESUME

Strategies de défense des Cochenilles : Inference phylogenétique et scenarios evolutifs (Hemiptères, Coccoidea)

La vie sédentaire des Cochenilles accroît leur vulnerabilite face à leurs ennemis naturels et aux conditions de l'environnement. Elles ont developpé au cours de leur evolution diverses strategies de defense, qui augmentent leurs chances de survie et leurs succes reproducteurs. Ces strategies sont analysees en distinguant parmi elles : 1. l'elaboration de structures de protection a partir de leurs secretions et/ou de leurs excretions ; 2. l'exploitation de l'effet protecteur offert par leurs plantes-hôtes ; 3. la modification de leur cycle évolutif en reponse aux facteurs environnementaux ; 4. la modification profonde du corps des femelles pour la protection de leur descendance. L'utilisation du microscope electronique a balayage ainsi que diverses experimentations, ont permis d'expliquer la formation de ces structures. Une analyse cladistique portant sur 25 genres a partir de 54 caracteres morphologiques a donne deux arbres equiparcimonieux. Cette phylogenie sert de reference pour etablir l'etat ancestral et les changements subsequents a la fois pour le choix de l'habitat et pour le type de structure de protection. L'analyse phylogenetique suggere que l'etat ancestral est equivoque et actuellement nous ne pouvons pas choisir entre un habitat aerien (tronc) et un habitat souterrain (racines). Il est plus parcimonieux de considerer la protection par des secretions amorphes comme l'etat ancestral. Les changements subsequents de l'habitat ont conduit a la conquete des branches.

FOLDI, I., 1997. — Defense strategies in scale insects: phylogenetic inference and evolutionary scenarios (Hemiptera, Coccoidea). *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mém. Mus. natn. Hist. nat.*, **173**: 203-230. Paris. ISBN: 2-85653-508-9

Source: MNHN, Paris

des feuillage des arbres et arbustes et des tiges de graminees La branche est la partie la plus exploitée des plantes-hôtes La protection a été tout d'abord assurée par des secrétions non organisées recouvrant le corps, puis par la construction de structures de plus en plus complexes, avec une tendance à trouver un compromis entre les dépenses énergétiques et l'efficacité

INTRODUCTION

The adult females of contemporary scale insects are neotenic and apterous and are adapted to a sedentary parasitic habit, sucking sap from their host plant's vascular tissue Not only have the Coccoidea colonized many plant families but they have also exploited all parts, with species living on the leaf sheaths, hypocotyl and roots of grasses and on the trunks, branches, leaves and fruits of shrubs and trees Scale insects are often an important component of phytophagous ecosystems, especially in tropical and subtropical areas and many species are injurious to cultivated plants, such as vines, palms, citrus, forest or ornamental trees and also to indoor plants They occur in all zoogeographical regions of the world and in a wide range of ecosystems, from dry desert to cold tundra, humid forest or high up mountains

These sedentary insects have evolved a series of original and effective protective structures ensuring their survival in these diverse environments, providing not only a favorable microenvironment with regard to temperature and humidity, but also some degree of protection against such natural enemies as predators (mainly Coleoptera) and parasitoids (primarily chalcidoid wasps) and also against chemicals and air pollutants.

The scale insects are undoubtedly a natural group and their evolution is particularly interesting because the Coccoidea display a remarkable diversity of often unusual structural and biological features However, the relationships of several family-group taxa, as well as the monophyly of some families remain controversial and not well established Several authors have attempted to estimate phylogenetic relationships within the Coccoidea but until now such estimates have been largely intuitive In these cases, the proposed phylogenies are not refutable and cannot be submitted to the test of adding new taxa and new characters A comprehensive and interesting phylogenetic analysis of Coccoidea has been carried out by DANZIG (1980) The following works DANZIG (1984, 1990), COX & WILLIAMS (1986), COX (1984), FOLDI (1984), KOTEJA (1974), BORATYNSKI & DAVIES (1971) and BORCHSENIUS (1956) used analytical methods other than cladistics and are, therefore, less easily supported MILLER & KOSZTARAB (1979) and KOSZTARAB (1996) presented phylograms of the intuitively modified cladograms of BORATYNSKI & DAVIES (1971) and DANZIG (1980), respectively The use of cladistic analysis has recently been developed among coccidologists to estimate sister-group relationships within the Coccoidea (MILLER, 1984, MILLER & MILLER, 1993a, 1993b, FOLDI, 1995, MILLER & WILLIAMS, 1995, QIN & GULLAN, 1995, HODGSON & HENDERSON, 1996)

In the present work, the adaptive defense strategies in scale insects were studied, and in order to test various scenarios for the evolution of the main habitats and protective structures for some of the main taxa of the Coccoidea, an independently reconstructed phylogeny was used as reference (CODDINGTON, 1988, CARPENTER, 1989, DEPORTE, 1993, GRANDCOLAS *et al.*, 1994, DESUTTER-GRANDCOLAS, 1994, MILLER & WENZEL, 1995, ANDERSEN, 1995, SCHULTZ *et al.*, 1996) These biological attributes (habitat and type of protective cover) have been mapped on a cladogram and polarized by optimization with principle of parsimony and it is hoped that this may provide a reasonable hypothesis of the ancestral state and its subsequent direction of historical transition

Source MNHN, Paris

MATERIALS AND METHODS

Porphyrophora chrithmi was reared in a greenhouse on *Crithmum maritimum* (Umbelliferae), whereas *Eurhizococcus brasiliensis*, *Margarodes cadeti*, *M. vitis* were reared on *Solanum tuberosum and Cucurbita maxima* and *Eriococcus buxi* on *Buxus sempervirens*. Cladistic analysis was implemented with the Paup 3.1.1 (SWOFFORD, 1991) and MacClade 3.05 (MADDISON & MADDISON, 1992) computer software packages. Character change analysis was performed by MacClade and the parsimony analysis of the character matrix with the "Heuristic search" algorithm of Paup with the "acctran" optimization. Multistate characters were treated as unordered and characters were unweighted. Unknown characters were coded as missing data. The size and structure of data matrix prevented the use of the exhaustive or branch and bound methods. The data matrix included 25 genera, representing each of the currently recognized scale insect families, with 54 characters from adult females, adult males and first instar nymphs. Data were gathered either from published information (GREEN, 1922, JAKUBSKI, 1965, RICHARD, 1986, TAKAGI, 1987, 1992, GIL, 1988, 1993, KOSZTARAB & KOZAR, 1988, WILLIAMS & WATSON, 1990, MORALES, 1991, MILLER, 1991, MAROTTA *et al.* 1995) or from direct examination of specimens as follows: *Matsucoccus feytaudi* Ducasse (Margarodidae), *Margarodes formicarum* Guilding (Margarodidae), *Stigmacoccus asper* Hempel (Margarodidae), *Carayonema orousseti* Richard (Carayonemidae), *Orthezia urticae* (Linnaeus) (Orthezиidae), *Phenacoleachia zealendica* (Maskell) (Phenacoleachiidae), *Conchaspis vayssierei* Mamet (Conchaspididae), *Planococcus citri* (Risso) (Pseudococcidae), *Eriococcus buxi* (Fonscolombe) (Eriococcidae), *Kermes vermilio* Planchon (Kermesidae), *Dactylopius coccus* Costa (Dactylopiidae), *Coccus hesperidum* Linnaeus (Coccidae), *Cerococcus quercus* Comstock (Cerococcidae), *Asterodiaspis variolosa* (Ratzeburg) (Asterolecaniidae), *Lecanodiaspis sardoa* Targioni-Tozzetti (Lecanodiaspididae), *Aclerda berlesi* Buffa (Aclerdidae), *Tachardia albizziae* Green (Tachardiidae), *Micrococcus silvestri* Leonardi (Micrococcidae), *Stictococcus intermedius* (Stictococcidae), *Phoenicococcus marlatti* Cockerell (Phoenicococcidae), *Halimococcus thebaicae* Hall (Halimococcidae), *Beesonia napiformis* Kuwana (Beesoniidae), *Chionaspis salicis* (Linneaus) (Diaspididae). The aphids (Aphidoidea), traditionally considered as forming the sister-group of the Coccoidea and recently confirmed by cladistic analysis of molecular data (SORENSEN *et al.*, 1995, VON DOHLEN & MORAN, 1995), were used as outgroup to determine polarities of characters (*Eriosoma* spp. (Pemphigidae) and *Myzus persicae* (Aphididae).

To test hypotheses regarding the evolution of particular biological attributes, this independently obtained cladogram was used as a reference system. The biological attributes (habitats, *i.e.* feeding sites on host plants, and protective structures) were then mapped on this cladogram. The scenario for their evolutionary changes was derived by optimization using the principle of Wagner parsimony and unordered states with MacClade 3.05 computer software.

TYPES OF DEFENSIVE STRATEGIES OF SCALE INSECTS

The strategies which have evolved to defend the bodies of scale insects and their progeny (eggs and newly hatched first-instar nymphs) can be divided into four major groups:

1. production of structures which are made from secretions and/or excretions which either adhere to the body or form amorphous waxy secretions, cysts or separate tests, this type of protection, from simple to complex, is the most widespread and the most characteristic of Coccoidea,
2. specific choice of settling site to exploit host-plant afforded protection (*e.g.* those which live beneath bark, under leaf-sheaths or on the nodes of grass stems, or which are gall formers),
3. modifications of the life-cycle in response to environmental factors, as found in the cyst-forming Margarodinae,
4. modification of the body of the female to provide a protective cover, such as by the formation of a marsupium, as in the Margarodids, or the heavy sclerotization of the dorsum of soft scales, in Kermesids or Stictococcines.

All Coccoidea are protected by one of the mechanisms described above. In some unrelated groups or in groups in which the protective mechanism has evolved convergently, it is probably dictated by habitat-required defense strategies. However, JASCHENKO (1993) observed that some Margarodids living in desert may utilize complementary protection and show particular ecological adaptations, *e.g.* the scale insects may form a buffer layer around their body or leave

Source: MNHN, Paris

the soil surface for a deeper ground layers Mutualistic relationships with ants may appear in some group as an alternative strategy for their protection These four categories will now be discussed in more detail

STRUCTURES CONSTRUCTED FROM SECRETIONS / EXCRETIONS PRODUCED BY SCALE INSECTS

Most scale insects secrete a series of external protective covers during each stage of their development These covers or tests regulate the temperature and control water loss, thus maintaining an equable microclimate beneath the test, so that, when the test is removed, the Coccoids die through desiccation The diverse methods which have evolved to protect the eggs have been illustrated recently by KOTEJA (1990)

The material used in the construction of these covers is secreted (i) by the well-developed wax gland systems typical of Coccoidea and also (ii) from other substances eliminated through the anus *i.e.* the final products of metabolism The range of integumentary glands which secrete the former are diverse and their secretions are transported to the body surface through specialized cuticular structures pores, ducts, ductules and secretory setae (FOLDI, 1991) The shape taken by these secretions is dictated by both their chemical composition and by the shape of these cuticular structures, which act as moulding devices These secretions are always mixtures, the two most common components being waxes and resins The relative amounts of these components determine the characteristics of each secretion which, in turn, determine the color and hardness of the protective covers These secretions also play an important role in the protection of the respiratory pathway and in assisting in honeydew elimination (FOLDI & PEARCE, 1985).

Protective covers constructed from anal fluids

Based on the materials used in the construction of the test or cover, it is possible to distinguish five types of protective cover, namely those constructed from· anal fluids, mixtures of filamentous wax secretions and anal fluids, mixtures of filamentous and amorphous wax secretions, exclusively from amorphous wax secretions, and exclusively from filamentous wax secretions

Cyst formation Both sexes of species belonging to the subfamily Margarodinae have an unusual postembryonic development that includes an atypical second-stage nymph, commonly referred to as the cyst stage (Figs 8, 10, 12) The nymph of this cyst stage is legless and is completely enclosed in a shell constructed from anal fluids by the insect The enclosed nymph has

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FIGS 1-7 **1**: Adult female *Kermes roboris* FOURCROY (Kermesidae) **2**: Two adult female tests of *Unaspis yanonensis* (KUWANA) (Diaspididae) **3**: Group of adult female *Lecanodiaspis sardoa* TARGIONI-TOZZETTI (Lecanodiaspididae) **4**: Adult female *Beesoma dipterocarpi* GREEN (Beesomidae) located under the bark of its host plant **5**. *Apiomorpha conica* (FROGATT) (Eriococcidae) showed the adult female in its gall **6**: A colony of adult female *Gascardia madagascariensis* TARGIONI TOZZETTI (Coccidae) The scale insects are engulfed in a thick yellow test composed of secretions + excretions **7**: Detail of FIG 6 showing a fragment of test with the included females.

Source MNHN Paris

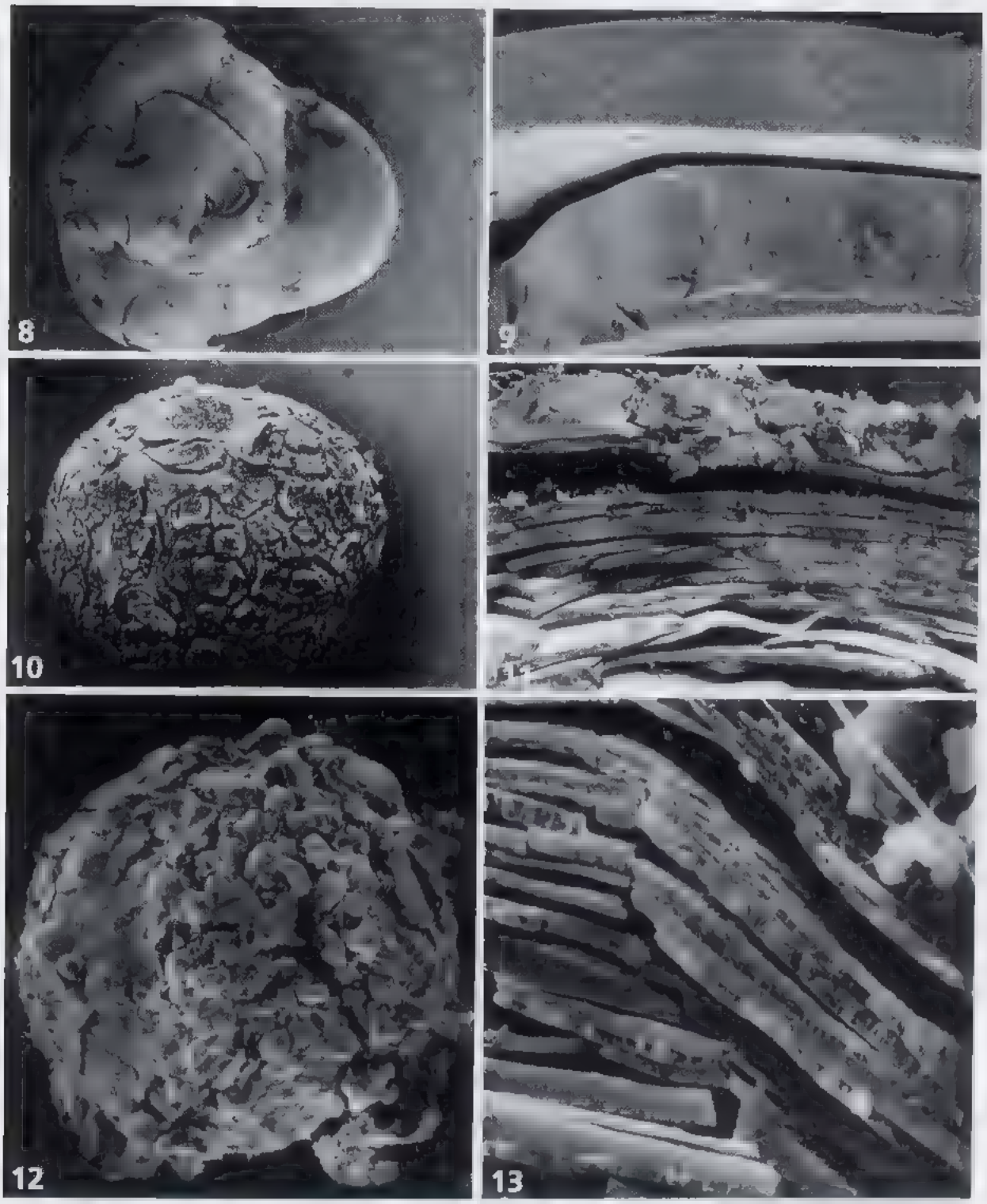
Source MNHN, Paris

well-developed, functional mouthparts and, in most cases, the body is large and globular (although it may sometimes be elongate) but it always contains a large quantity of fat body. When the host plant lives in good environmental conditions (rain and sun assured), this cyst stage can last from several months to about a year, depending on the species. However, under unfavorable ecological conditions (*e.g.* prolonged dry period causing the death of the host plant), the cyst stage may be extended to several years. The second moult occurs within the cyst, giving rise to the adult female, which again possesses legs and antennae. Emergence of the adult female from the cyst is by first using the anterior legs to make a small opening in the shell wall and then squeezing through to exterior. This is possible due to the great flexibility of the body. In bisexual species, the adults then migrate to the soil surface to mate, and after this the females move back down into the soil and oviposit on or near the roots (FOLDI, 1990a).

The function of these cysts or shells has long remained unclear, mainly because the anatomy of the nymphs has not been well studied and because the structure and the processes of cyst formation have not been understood. The structure and formation of the cysts of *Margarodes formicarum*, *M. vitis*, *Eurhizococcus brasiliensis* and *Porphyrophora crithmi* were therefore studied, both experimentally and by the use of the scanning electron microscope (Figs 8-13). In most cases, the young first-instar nymphs settle on the host plant with their head down and their anus up. After settling and feeding, the secretion starts from the spiracular wax glands, while droplets appear from the anus and these slowly flow around the posterior end of the body and solidify (FOLDI, 1981). Sometimes several droplets are expelled in rapid succession. Even where the nymph settles in an oblique or horizontal position, the anal liquid flows over the body in the same way. The continuous feeding produces a rounded body and the anal liquid, which is regularly extruded, forms more and more overlapping layers on the body surface. If the anus is cauterized at this stage, the production of the anal liquid ceases and the construction of the shell stops. Alternatively, if the outer shell is removed experimentally, droplets continue to appear from the anus and shell formation commences once again, with the body gradually becoming covered with fine layers of secretion, the cyst finally becoming entirely enclosed. Electron microscope studies have confirmed the existence of 5 to 30 or more overlapping layers, each layer composed of an amorphous substance of equal or varying thickness, ranging from 5 to 100 µm thick (Figs 9, 11, 13). Thus, it appears that the structure of the cyst or shell is made exclusively from anal liquids eliminated by the nymph. The hardness, colour, size, shape and external surface morphology of these cysts varies considerably from one species to another. The hardness and colour are determined by the final metabolic products, and these are linked to the chemistry of the sap of the host plant and perhaps also to the secretions of some currently unknown glands located in the digestive system. In addition, the external surface of the cyst may include many extraneous particles, such as sand, earth or pieces of vegetation and these particles may completely mask the protective tests.

FIGS 8-13 - **8-9**: Cyst stage of *Margarodes formicarum* Guilding (Margarodidae). The cyst wall is formed by numerous overlapping layers of anal fluid. **10-11**: Cyst stage of *Margarodes vitis* (Philippi) (Margarodidae) with the detail of its wall structure. **12-13**: Cyst stage of *Porphyrophora polonica* Linnaeus (Margarodidae) and the detail of its wall showing the layered structure.

Source: MNHN, Paris



Source MNHN Paris

These cysts are generally located on the roots or collar of their host plants and are also referred to as "ground pearls" This name comes from their external appearance and their habitat. They are found in the sand or soil and are hard, round and pearl-like, often shiny and colorful, even sometimes slightly iridescent There are ethnozoological reports of ground pearls being used for human utility Like true pearls, ground pearls are strung on a filament and sold as necklaces in Bermuda and neighboring islands, in the Mediterranean area and wherever probably *Margarodes* pearls are found

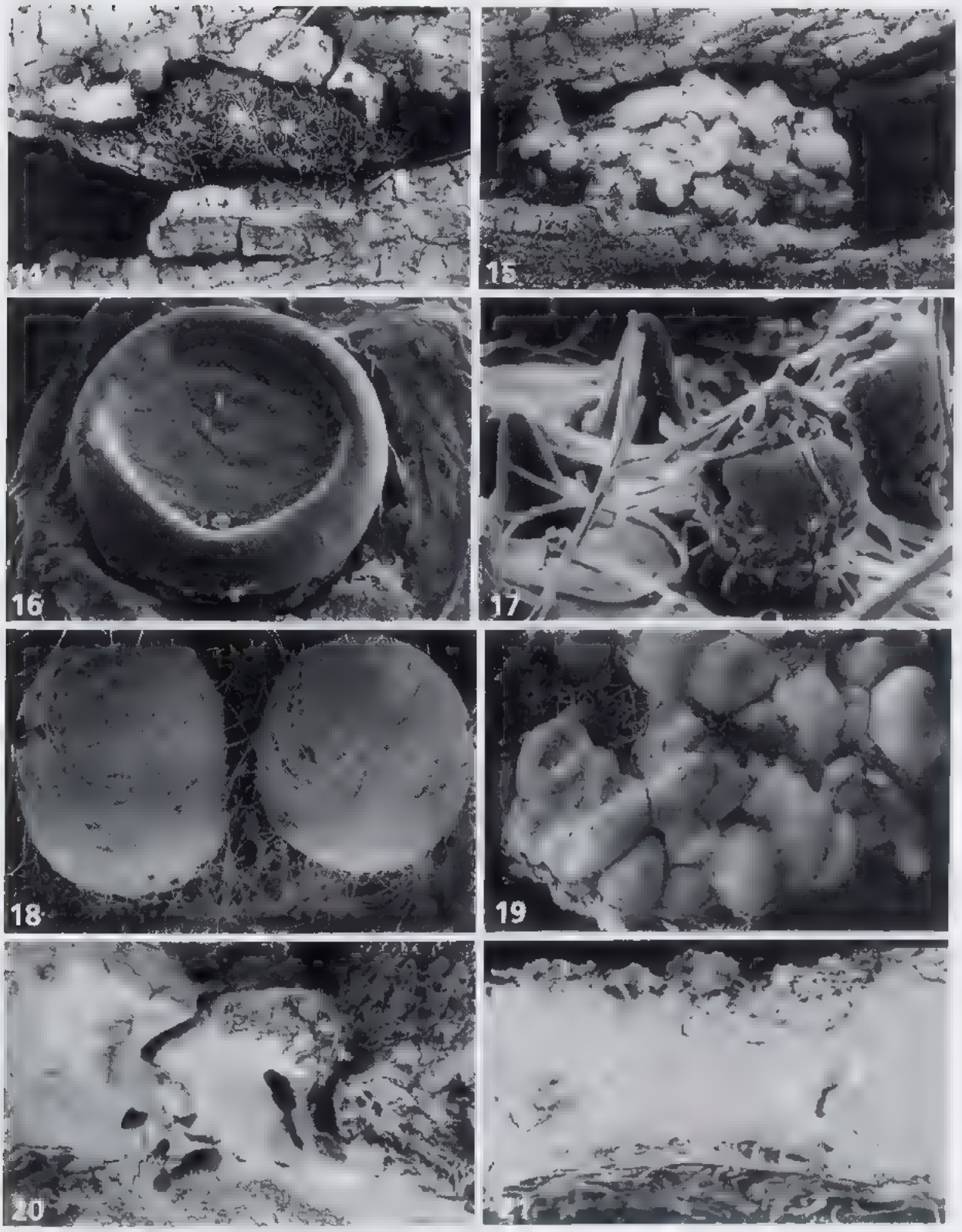
Protective tunnel formation Anal fluids are also used by *Limacoccus* spp (Beesoniidae) for the construction of their protective tunnel In this genus, after the first moult, the second-instar females are located inside the exuviae The produced anal fluid is pushed forward by the body's contraction and it is extruded through the exuvial split to the exterior By the movement of the anterior part of the body, the nymph forms a semicircular tunnel in the anal substance which solidifies quickly The nymph advances progressively in this tunnel and settles outside for the second moult The wall of the tunnel is composed of numerous hard layers (FOLDI, 1995a)

Structure and formation of separate tests using filamentous wax secretions and anal fluids The scale covers of the Diaspididae represent the most elaborate type of protective structure whose physical properties provide an effective barrier They are formed from a combination of waxy filaments, mostly secreted by pygidial glands, which are cemented together by the successively extruded anal fluid, and formed either in a circular or an elongated shape by the movements of the body The exuviae of previous instars are also incorporated into these covers (Fig 2) Cover formation and how such factors as cauterization of the anus, delayed mating or insufficient food interfere with its construction are described in detail by FOLDI (1982, 1990b, 1990c) The cover formed by the Conchaspididae is constructed in a similar manner, except that it does not incorporate the exuviae and the anal secretions are not distributed actively over the dorsum (FOLDI, 1983).

Structure and formation of separate tests using filamentous wax secretions and amorphous secretions Such species as *Cryptokermes brasiliensis* Hempel (Margarodidae), *Stigmacoccus asper* Hempel (Margarodidae) and *Ultracoelostoma assimile* (Maskell) (Margarodidae) form a protective test in which all stages of development are enclosed The structure and the process of formation of the protective test was studied in *Cryptokermes brasiliensis* using SEM (Figs 14-21) The first-instar nymphs settled in crevices in the bark of *Vitis* sp and started feeding Waxy secretions were produced by multilocular wax glands which covered all the body, these were either short, curved secretions or long filaments (Fig 14)

FIGS 14-21 **14-15:** First-instar nymph of *Cryptokermes brasiliensis* Hempel (Margarodidae) The filamentous secretions covering the body is completed by a series of soft ball shaped secretions **16:** Dorsal cicatrix of *Cryptokermes brasiliensis* Hempel (Margarodidae) **17:** Large spine-like structures with their amorphous secretions on the dorsum of *Cryptokermes brasiliensis* Hempel (Margarodidae) **18-19:** Detail of the coalesced ball shaped amorphous secretions among the filamentous secretion of *C brasiliensis* Hempel (Margarodidae) **20-21:** Section of the cyst stage of *Cryptokermes brasiliensis* Hempel (Margarodidae) showing its wall structure

Source MNHN Paris



Source MNHN, Paris

Simultaneously, the perianal and anal wax glands started to produce a waxy anal tube which served to eliminate the honeydew This tube reached twice the length of the 1st-instar body after only about 24h Amongst this tangled, loose network of secretions some small, soft, ball-shaped amorphous secretions appeared which originated (i) from numerous cicatrices scattered on the dorsum and (ii) from large spine-like structures scattered between the cicatrices (Figs 15, 19) These amorphous secretions were scattered over the body and hardened slowly They gradually increased in size and number so that they finally coalesced together The shape of test covering the 1st-instar nymphs was fusiform, corresponding to the shape of the nymph's body, although the dimensions of the internal cavity was larger than the size of the body After the first moult, the test (which was not completely finished yet) became more and more globular. The 2nd- and 3rd-instar nymphs continued to add new secreted material to the wall of the test, increasing its thickeness By the time the insect was adult, the test was 5-6 mm in diameter and had a very rough external surface which was covered with bumps In transverse section, the test wall was 0 5-0 6 mm thick and appeared to have an external layer formed from the ball-shaped amorphous secretions and an inner layer which was an amorphous mass (Figs 20-21) The test was very hard and the final colour was black The adult females remained enclosed in this test and mating occurred through a small opening left by the anal tube of the immature stages, this aperture was also used by the newly hatched nymphs for dispersal *Lecanodiaspis sardoa* Targioni-Tozzetti (Lecanodiaspididae) (Fig 3) constructs a protective test which is formed from long filaments, secreted by the tubular duct wax glands, which are cemented together by amorphous secretions produced by the cribriform plates and the 8-shaped pores (FOLDI & LAMBDIN, 1995)

Structure and formation of separate tests using exclusively amorphous secretions or mixture of amorphous secretions and anal fluid There are two types of amorphous secretions, the first is produced by the spine-like setae in Ortheziidae and the second by a variety of different wax gland systems found in the remaining scale insect taxa In the majority of soft scales, the dorsal integument is covered by an amorphous and layered secretion, which increases the thickness of the dorsum and assists in its resistance to unfavorable external factors In *Inglisia vitrea* Cockerell (and other Cardiococcinae), the cover is composed of a glassy, transparent covering composed of resin This test is comparable to that found in the Asterolecaniidae, in which the test enclosing the female is also amorphous, hard and transparent In *Cerococcus quercus* Comstock (Cerococcidae), the test of the adult females is an amorphous, yellow, smooth structure, although it has long waxy filaments on the inside When the populations of these scales are dense, the tests fuse together to form a large mass on the twigs The exuded mixture of amorphous secretions and anal fluid is so abundant in *Kerria lacca* (Tachardididae) and *Gascardia madagascariensis* (Coccidae) that it engulfs the entire colony forming the characteristic "stick secretions" on the twigs (Figs 6-7)

Structure and formation of separate tests using exclusively filamentous secretions The use of only filamentous secretions for their protection is found on the adult females of many Margarodinae, Eriococcidae and on such soft scales as the Eriopeltinae or Filippinae The filamentous secretions forming either a loose network or a more structured test but it covers always some or all of the dorsal surface of the female

Source MNHN Paris

Ovisac-like structures After mating, the females of several groups (some Eriococcines, soft scales and Margarodids) produce filamentous secretions that enclose the female's body and also her eggs and newly-emerged nymphs Other species construct a specific structure, the ovisac, which exclusively encloses the eggs and newly hatched nymphs Thus, we can distinguish ovisac-like structures that enclose the female plus her eggs and nymphs from an ovisac constructed exclusively for protecting eggs and nymphs The ovisac-like structure is found in female *Eriococcus buxi* (Fonscolombe), (Eriococcidae) This species construct a separate, felted sac-like test which encloses the body and eggs, except for a small hole at its posterior end When observed by SEM, the wall of this test is made exclusively from a dense network of waxy filaments secreted by the tubular duct wax glands Within this test, the eggs are covered by a loose network of short waxy filaments secreted by the ventral multilocular disc-pores These protective tests cover the whole of the female's body and also the eggs and are composed of a loose network of filamentous waxy secretions This ovisac-like structure cannot be considered as homologous to a true ovisac For instance, mated female Margarodinae secrete by the multilocular wax glands distributed on the entire body a loose network of long waxy filaments, which encloses both the adult female and eggs However, in addition, short curved filaments are secreted by the wax glands of the same type but with inclined loculi, that are distributed around the vulva, and these filaments cover the surface of the eggs In some species, such as those in the genus *Gossyparia* (Eriococcidae), the filamentous secretions which protect the eggs only partially cover the adult female along the margins

Ovisac Many females Coccoids protect their eggs during embryonic development and then their newly hatched crawlers by constructing a true ovisac Several unrelated groups of Coccoidea such as the iceryine Margarodids, Ortheziids, Pulvinarine soft scale, Kermesids – *e.g. Nidularia pulvinata* (Planchon) and many mealybugs secrete a white ovisac These ovisacs are characteristic structures, secreted by divers wax glands, which are clearly produced from beneath the posterior end of the abdomen of the adult females and are often ornate and have a characteristic shape The presence of this type of ovisac in such a wide range of Coccoidea suggests that they evolved convergently In groups with tubular duct wax glands, these are considered to secrete the external part of the ovisac, as in pulvinarine, filippiine and eriopeltine soft scales (HODGSON, 1994), indeed the Pulvinariini generally have three or four different types of ventral tubular ducts and also produce the most complex ovisacs within the Coccidae, in terms of the types of wax making up their ovisac (HODGSON, personal communication) In the simplest cases, the ovisacs are composed of two types of secretions (i) an outer layer made of long filamentous waxy secretions which are thoroughly cemented together and which form a solid outer layer, and (ii) an inner layer composed of short, generally curved, waxy secretions which form a loose network around the eggs In other taxa, such as in some soft scales species numerous types of wax glands may participate in its construction An exception is found in the Ortheziids, in which the external part of the ovisac is made of (a) amorphous waxy secretions produced by the spine-like setae located in a large submarginal band on the abdomen and (b) by the long, thin filaments produced by the quadrilocular pores on the inner part The construction of these ovisacs only starts after mating and is often finished whilst females are still laying The best known example is the cosmopolitan *Icerya purchasi* Maskell which secretes an ovisac often

Source MNHN, Paris

longer than its body, in which the hard, ornate and beautiful outer part is produced by two types of ventral wax glands externally on the body margin are the large open centred pores and internal to these are the multilocular pores which form a broad submarginal band around the abdomen The inner, looser part of the ovisac (which encloses the eggs) is secreted through the multilocular disc-pores around the vulva area The red colour of the newly hatched 1st-instar nymphs, with black legs, are clearly visible within this white, cottony sac

BEHAVIORS WHICH HAVE EVOLVED TO EXPLOIT PROTECTION AFFORDED BY THE HOST PLANT

Many Coccoids settle in confined, protected feeding sites provided by their host plants, such under leaf-sheaths, leaf-axils, nodes of grass, in crevices or under bark, and on roots and root-crowns This behavioral adaptation to protected feeding sites may be observed in most higher taxonomic groups in the Coccoidea, however the largest diversity is found in the Margarodidae *s.l.*

Species which live beneath bark or in bark crevices

Species of the genera *Kuwania*, *Steingelia*, *Neosteingelia*, *Xylococcus* and *Xylococculus* live under the bark of such trees as *Pinus*, *Prunus* and *Quercus Matsucoccus* spp live in cracks and crevices or under the bark of *Pinus* spp In these taxa, the cyst stage secretes a glassy test (as described above), while the adult females secrete white woolly waxen threads over the body

Species which oviposit beneath bark and otherwise live on the leaves Stomacoccus platani Ferris lays its eggs beneath the bark on the trunk of the host tree The newly-hatched 1st-instar nymphs emigrate to the undersurface of the leaves When fully grown, the mated females migrate back to the trunks and lay the eggs beneath the bark.

Species which live beneath leaf-sheaths or on the nodes of grass stems Several eriococcine, pseudococcine and coccine species live under the leaf-sheaths or on the nodes of grass stems Their bodies tend to be flattened dorso-ventrally and are only weakly covered by waxy secretions, although the body of *Aclerda berlesi* Buffa (which lives under the leaf-sheaths on the stems of *Arundo donax*) is surrounded by a large amount of glassy secretion However, the body of *A. berlesi* is also flattened dorso-ventrally and becomes strongly sclerotized in old females

Gall formation Galls are products of host-insect interactions and are a response by the plant to chemical stimuli from salivary glands of the gall former The gall-forming arthropod benefits in having an improved food supply, good environmental conditions and reasonable security Extensive studies have been made on the gall-forming Coccoidea by GULLAN (1984a,b,c) and BEARDSLEY (1984) The majority of gall-forming scale insects belong to the Eriococcidae, which are abundant in Australia, principally on *Eucalyptus* Galls formed by adult females of *Apiomorpha* Rubsaamen (Fig 5) have species-specific morphology and vary greatly in size and shape, from cylindrical to globose and sometimes with arm-like extensions (GULLAN, 1984a,b) Galls of *Apiomorpha* are also sexually dimorphic, with the galls of males being much smaller than those of females There are only two gall forming Margarodids currently known, *Matsucoccus gallicolus* Morrison and *Araucaricoccus queenslandicus* Brimblecombe With *M.*

Source MNHN Paris

gallicolus, which is a pest on pitch pine, the feeding activity of the crawler causes the host-plant tissues to collapse, forming a depression beneath its body By the time it moults to form the cyst stage, about six to nine weeks later, the host-plant tissue has grown to completely cover the Margarodid, except for a small hole Once adult, the females generally disperse through this hole to the outside, although sometimes the hole is too small and the female is then obliged to stay and oviposit within the gall

ADAPTATIONS OF THE LIFE-CYCLE TO AVOID ADVERSE ENVIRONMENTAL CONDITIONS

The cyst of some species of *Margarodes* is highly resistant to adverse environmental conditions, which they are capable of surviving for a long time, only undergoing their last moult when conditions are again favourable Prolonged absence of rain provokes the death of the host plant and, when again it rains, this water humidifies the soil around the cysts, which initiates their moult MAYET (1896) noticed that the cysts of *Margarodes vitis* (Philippi) emerged as adult females once they had been immersed in water, even though they had been kept without food for seven years, while FERRIS (1919) reported an adult *M. vitis* emerging after seventeen years of storage Like *M. vitis*, species which are capable of surviving such long periods have a highly resistant shell, with a thick and very hard wall Similar observations were made by DE KLERK *et al.* (1980) on *Margarodes capensis* when females detached from their host plants under laboratory conditions could emerge during four successive years My personal observations on the cysts of several *Margarodes* spp is that their long-term survival is assured only for the nymphal stages and not for the adult females, even if the latter stay inside the cyst The adult female of the cyst-forming species also have the ability to move from an unsuitable habitat to a suitable one, which is a characteristic that most scale insects have lost

This protection afforded by the cysts is particularly important when the species are injurious to cultivated plants For example, the cyst-forming Margarodids *(Eurhizococcus brasiliensis* and *Margarodes vitis)*, injurious to vineyards in South America, are able to resist most control measures, including soil-applied insecticides (FOLDI & SORIA, 1989) This ability of the cyst-forming species to survive unfavorable conditions is almost certainly more widespread than we currently know but our observations are mostly limited to those species which are of economic importance

PROTECTION PROVIDED BY MODIFICATIONS OF THE BODY OF THE FEMALE

Alternative strategies for protection have been developed by some groups of Coccoids where the body of the adult female has become modified to provide protection, either by the ventral surface becoming invaginated to form a marsupium, as in several Margarodids, or by the dorsum become strongly sclerotized, as in Kermesids, many soft scales and Stictococcids

Formation of a marsupium

Although plesiomorphic in condition, the marsupium is the most remarkable invention for protecting the eggs and crawlers Based on the method of formation of the marsupium, we can distinguish two types (i) the internal marsupium is formed by a deep invagination of the integument within the body to form a large cavity, whereas (ii) the external marsupium is formed by depression of the ventral integument and the resulting cavity is covered by a secreted

Source MNHN, Paris

operculum An internal marsupium is found in a number of unrelated genera of Margarodids, *e.g.*, *Callipappus*, *Steatococcus* and *Etropera* spp After mating, a deep invagination of the integument appears in the teneral female, either ventrally or in the posterior part of the abdomen, forming a cavity (MORRISON, 1928) Initially, the cavity is small but it progressively deepens during egg-laying It is normally restricted to the abdomen but can extend even into the cephalothorax area In species of *Steatococcus*, *Etropera* and *Perissopneumon*, the cavity is formed by the invagination of the sterno-abdominal integument The vulva opens dorso-posteriorly into the marsupium and the eggs are laid directly into it In *Callipappus* spp., this invagination is at the posterior end of the body, but can extend anteriorly within the body cavity as far as the head, as in *Callipappus westwoodi* Guérin-Meneville in which the long, narrow vagina opens antero-dorsally through the vulva into the marsupium

The external opening of the marsupium in different taxa is highly variable In *Steatococcus* spp., the opening is circular and is placed medially near the metathoracic legs, surrounded by numerous multilocular wax glands, in *Etropera* spp (BHATTI & GULLAN, 1990), the opening is large, forming an arc, whose outer margins lie near the metathoracic legs but which lack wax glands, while in *Callipappus* spp the opening is also large but is elongate and located at the posterior end of the body In females possessing an external marsupium, a part of the ventral abdominal region containing the vulva becomes depressed and is then isolated from the exterior by an operculum composed of wax secreted by a large band of glands which surround the cavity These glands produce large quantities of waxy filaments which become cemented together by an amorphous secretion These opercula are solid, resistant to outside pressures and very hard to detach from the body The eggs are laid inside this protective cavity and, once the nymphs have hatched, they leave through a small opening at the anterio-medial part of the operculum There is considerable diversity in the size of these pseudo-marsupial pouches and their opercula In species of *Aspidoproctus* and *Hemaspidoproctus*, the cavity is small, located in the central part of the abdomen and the operculum is thick and resistant In *Gigantococcus maximus* (Newstead), the cavity is about 3 mm deep, incorporating most of the ventral surface of the abdomen, but with a rather softer and thinner operculum (BIELENIN, 1971)

Using the dorsal surface of the body as the protective cover

In this group, the dorsal cuticle of the female becomes thickened and strongly sclerotized at maturity, whereas the ventral surface remains thin and often becomes deeply invaginated, forming a cavity under the body which becomes a brood chamber used for holding the eggs and the crawlers This method of protection is typical of the Kermesids, many soft scales *e.g.* species of *Saissetia*, *Coccus*, *Parthenolecanium* and many others (HODGSON, 1994) and the Stictococcids (Fig 1) However, other soft scales secrete a thick waxy cover (*e.g. Ceroplastes* spp.) but, despite this, the dorsum becomes heavily sclerotized at maturity and the venter deeply invaginated, forming the brood chamber The eggs and crawlers are protected also under the body of *Aulacerya acaciae* Morrison & Morrison (Margarodidae) (GULLAN, 1986) LOWER (1957) reported an interesting example in the Pseudococcidae Species of *Epicoccus*, which live in arid conditions in Australia, develop a thickened and chemically modified dorsal integument, which is considerably expanded in comparison to the venter, thus offering excellent protection of the female and her progeny against the drying winds, high temperature and low relative humidity of their environment

Source MNHN Paris

Protection by ants

Facultative mutualistic relationships exist between several groups of scale insects and ants This relationship is of reciprocal benefit because for the ants the sugar-rich honeydew represents a food supply, and for the Coccoids the ants provide some protection against natural enemies, *i.e* predators and parasitoids Furthermore, ants by removing honeydew, reduce contamination of the Coccoids and so stop fungus development, which is injurious to the host plant and the scale insects Further protection may provided by ants that construct protective covers over Coccoid aggregations (FOLDI, 1984) Recently, WILLIAMS (1978) and HODGSON (1994) have described some deep morphological modifications, *e.g.* spiracular and anal adaptations, characterizing scale insects living an intimate relationships with the ants in their nests

PHYLOGENY

Currently, cladistic phylogenetic hypotheses for the entire Coccoidea are lacking, since only keys and classification are available to show the relationships of the higher taxa within the Coccoidea This cladistic analysis presents one of the first attempts to produce a preliminary estimate of the Coccoid phylogeny The genera used are representatives of the traditionally recognized families, however these families may not necessarily be monophyletic

Character and character state definitions

Adult female

1. Locular pores. (0) absent; (1) present
2. Wings· (0) present, (1) absent
3. Dorsal tagmosis: (0) distinct; (1) indistinct
4. Cornicles (0) present, (1) absent
5. Abdominal spiracles· (0) present; (1) absent
6. Ostioles: (0) absent, (1) present
7. Cicatrix-like structures: (0) present (1) absent
8. Legs· (0) well developed, (1) reduced or absent
9. Eight-shaped tubular ducts (0) absent, (1) present
10. Tubular ducts (0) absent; (1) not invaginated; (2) invaginated
11. Microtubular ducts· (0) absent; (1) present
12. Pores with thoracic spiracles: (0) absent, (1) present
13. Spiracular pore rows. (0) absent, (1) simple row; (2) double rows
14. Location of anus· (0) postero-dorsum or posterior, (1) middle dorsum or venter
15. Translucent pores on hind legs: (0) absent, (1) present
16. Dermal papillae near spiracles (0) absent, (1) present
17. Anal plates. (0) absent; (1) simple, (2) double
18. Anal opening (0) simple opening, (1) anal ring, (2) anal ring with setae and pores, (3) anal ring with setae
19. Mouthparts· (0) present; (1) absent
20. Number of instars. (0) 5 instars; (1) 4 instars; (2) 3 instars
21. Tarsal segments: (0) 2 segments, (1) 1 segment

First instar.

22. Antennal segments· (0) 6 segments, (1) 7; (2) 5 - 3

Source MNHN Paris

23 Apical antennae setae (0) few hair-like setae, (1) seate with a single spine, (2) hair-like setae with stout setae; (3) numerous hair-like setae (4) hair-like setae with fleshy seta or setae
24 Pores with thoracic spiracles (0) absent, (1) on meso + metathoracic, (2) only mesoth
25. Astero-type 8-shaped pores: (0) absent, (1) present
26 Bilocular pores (0) absent, (1) present
27. Trilocular pores. (0) absent, (1) present
28 Simple pores (0) absent; (1) present
29. Tarsal campaniform pore (0) present; (1) absent
30. Tarsal digitules. (0) absent, (1) present
31 Number of claws· (0) 2 claws, (1) 1 claw
32 Claw digitules: (0) absent, (1) present seta-like; (2) knobbed
33. Denticle on claw. (0) absent, (1) present
34 Tibia and tarsus length (0) ti > ta, (1) ti = ta, (2) ta > ti
35. Enlarged setae· (0) absent; (1) present
36 Femoral setae of hind legs: (0) several , (1) 1 seta or absent
37. Tibial setae of hind legs (0) several , (1) 1 seta or absent
38. Tarsal setae of hind legs· (0) several , (1) 1 seta or absent
39 Setae on legs· (0) abundant on all segment; (1) frequent, (2) few
40 Long caudal setae. (0) absent, (1) present
41. 8-shaped tubular ducts on the head (0) absent, (1) present
42. Labial segments· (0) 4 segments; (1) 3 segments, (2) 1 or 2
43. Labial setae (0) 12 or more, (1) 7 - 11, (2) 6 or less
44 Quadrilocular pores: (0) absent; (1) present
45. Quinquelocular pores. (0) absent; (1) present

Adult male
46. Compound eyes: (0) present; (1) absent
47. Number of simple eyes (0) absent, (1) 16, (2) 14; (3) 10: (4) 4
48 Antennal segments (0) < 9 segments; (1) 9 segments, (2) 10 segments
49 Abdominal spiracles: (0) present, (1) absent
50 Hindwings: (0) present; (1) present as hamulohalteres, (2) absent
51. Hamulohaltere setae: (0) absent, (1) 3-4; (2) 2;
52 Lateral view of aedeagus· (0) curved, (1) straight
53 Postocular ridge (0) dorsally weak or absent, (1) dorsally well present
54 Dorsal pore clusters on abdomen (0) absent, (1) one median on VI and VII, (2) one median on VIII, (3) two separated clusters on VIII

Analysis of the character data in Table 1 with the heuristic search algorithm of Paup resulted in two equally parsimonious cladograms The strict consensus tree in Figs 22-23 (Tree length 190, Consistency index (CI) 0 40, Retention index (RI) 0 59) shows some unresolved relationships among the genera The two trees differed in the placement of Coccus, which either was placed with the pit scales (*Astero-Cero-Lecanodiaspididae*) or was part of the group of relationships among the genera The two trees differed in the placement of Coccus, which either *Aclerda* + *Tachardia* The placement of *Micrococcus* is also problematic since it is the sister group to the *Stictococcus*+ According to MILLER & WILLIAMS (1995), the Micrococcidae is most closely related to the Aclerdidae *Stigmacoccus* (Margarodidae *sensu* MORRISON, 1928) is the sister group of all other scale insects and is characterized by two following autapomorphies anal ring (18 1) and two hamulohalteres setae (51 2) *Matsucoccus* + *Margarodes* (Margarodidae *s.l.*) are characterized by the following synapomorphies of the 1st instar nymph

Source MNHN, Paris

TABLE 1 — Character matrix used for the cladistic analysis of the Coccoidea

	1111111111222222222233333333334444444444555555
	1234567890123456789012345678901234567890123456789012345678901234
Eriosoma	000000000000000000000000000000000000000000000000000000000000
Myzus	000000100000000000000000000000000000000000000000000000000000
Stigmacoccus	111100000000000001020000000000110200000002000000012003
Carayonema	111100000000000003021210000000110200000002000??????????
Matsucoccus	111100000000000000120000000010120201102102200002011012
Margarodes	111100100000000000121220000010100201002102200?????????
Orthezia	111100100000000003011010000001111110001001010001011013
Phenacoleachia	111111100000000003011130000001121110000000000112010013
Conchaspis	111110100000000000021000000011121?011121011001401100l0
Planococcus	111111100100001003011030001011120200001101000142110003
Eriococcus	111110100211001003021030001011121210001101001141110013
Kermes	111110110200000001027040010001121211002101001132110013
Dactylopius	111110100200000002021020000011120210002001001142127013
Asterodiaspis	111110110101100002027041101101110200102102100142127010
Cerococcus	111110110201200003027021100101121200002101001142120010
Lecanodiaspis	111110110201100003027011101101121200002102101142117110
Stictococcus	111110100111110002021240000001120000002102000140127113
Coccus	111110100201100023021041001111121100002102211142127110
Micrococcus	111110100201000023011241010111120000001102000100127070
Aclerda	111110110101000012017031000111121010002102101142127000
Tachardia	111110110201000013027032000011121001002102201142127110
Phoenicoccus	111110111101010102027041000001121101112112201140127070
Halimococcus	111110111100000101027012000001120001112112200140127070
Beesoma	111110111101000001017231000011121201112112110140110170
Chionaspis	111110111101000000027242001001120201112112200142110110

tarsal campaniform pore (29 1), femoral setae on hind legs (36 1); setae on legs (39 1), long caudal setae (40 1) and absence of the mouthparts in adult females (19 1) However, the family Margarodidae appears to be paraphyletic because of the position of *Stigmacoccus* on the tree and the Margarodidae should be restructured into monophyletic groups A cladistic analysis of the Margarodidae *s.l.* and related groups is currently undertaken by the author (FOLDI, unpublished) In the remainder of the tree, the monophyletic family Carayonemidae is the sister group of the other Coccoidea and it is characterized by one autapomorphy, apical antennae with a single spine (22.1)

The autapomorphies representing cladistic diagnoses of the scale insects genera are as follows *Orthezia* setae frequent on legs (39 1), presence of quadrilocular pores (44 1), 9 antennal segments (48 1) and presence of 3-4 hamulohalter setae (51 1) *Phenacoleachia* Ostioles present (6.1), 6 antennal segments (22 0), reversal and, labial segments (42 0), reversal *Conchaspis* femoral seta1 on hind legs (36 1), tibial seta on hind legs (37 1), tarsal seta on hind

Source MNHN Paris

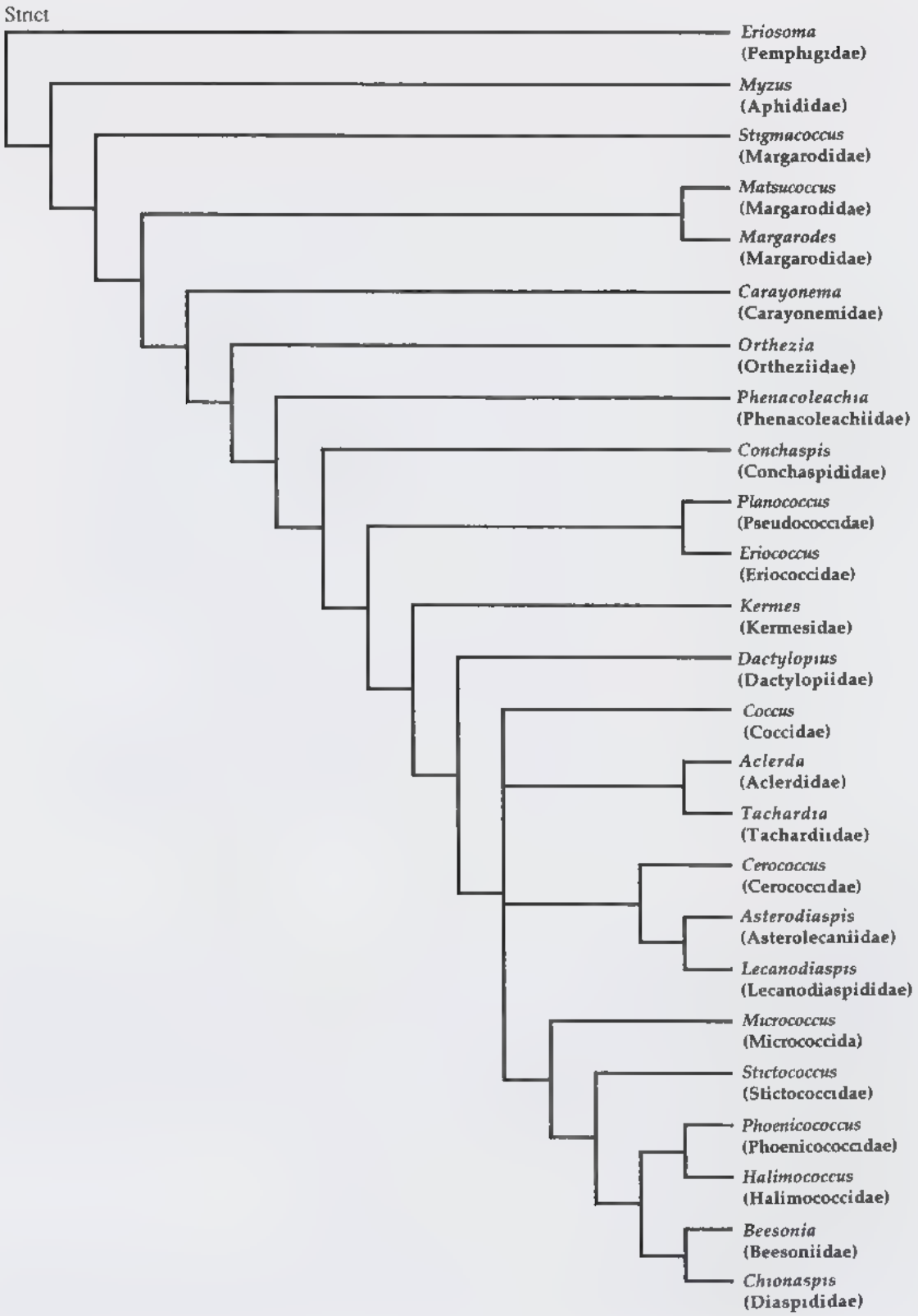


FIG 22. — Strict consensus tree showing the hypothetical relationships within the Coccoidea.

Source MNHN, Paris

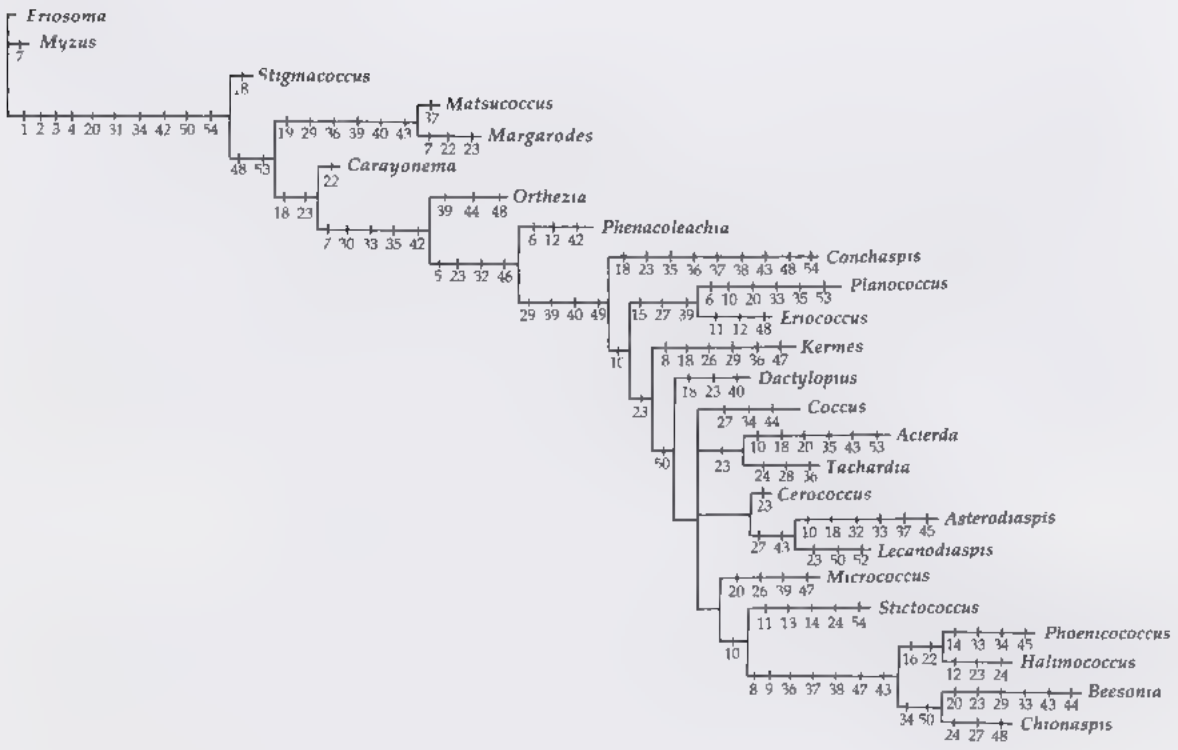


FIG 23 — Strict consensus tree with the character numbers

legs (38 1), labial setae (43 1) *Planococcus* and *Eriococcus* group is characterized by the following synapomorphies presence of translucent pores on hind legs (15 1), trilocular pores present (27 1), setae on legs frequent (39 2) *Kermes* presence of anal ring (18 1), legs (8 1), femoral setae on hind legs (36 1) and the number of simple eyes (47 3) *Dactylopius* anal ring with setae and pores (18 2) and apical antennae setae (23 2) The *Cerococcus* + *Asterodiaspis* + *Lecanodiaspis* group is supported by one apomorphy, the presence of the Astero-type 8-shaped pores The *Coccus* + *Aclerda* + *Tachardia* are characterized by two synapomorphies anal plates 17 1 and 2) and labial setae (43 1 and 2) *Micrococcus:* number of instars (20 1), bilocular pores (26 1) and setae on legs (39 1) *Stictococcus* presence of microtubular ducts (11 1), presence of spiracular pore row (13 1), location of anus on the middle dorsum or venter (14 1) and two separated dorsal pore clusters on VIII (54 3) *Phoenicococcus* + *Halimococcus* are supported by one synapomorphy dermal papillae near spiracles (16 1) *Beesonia* + *Chionaspis* are characterized by two synapomorphies tarsus longer than tibia (34 2) and the hamulohaltere are presents (50 1)

Some nodes are supported by a few characters, such as the node relating *Planococcus* + *Eriococcus* to *Kermes*+ is supported by one character, however there is a strong apomorphy, namely the presence of tubular ducts (10 1-2) The node relating the *Coccus* to Aclerda+ is also supported by a strong apomorphy, the absence of the hamulohalteres (50 2) In contrast, some other nodes are well supported, for example the *Orthezia to Phenacoleachia* + is supported by

Source MNHN Paris

five characters absence of cicatrix-like structure (7 1), presence of tarsal digitules (30 1), presence of denticle on claw (33 1), presence of enlarged setae (35 1) and the labium with 3 segments (42 1) The node relating *Phoenicococcus* to *Halimococcus*+ is supported by seven characters legs are reduced or absent (8 1), eight-shaped tubular ducts (9 1), femoral setae on hind legs (36 1), tibial setae on hind legs (37 1), tarsal setae on hind legs (38 1), eight-shaped tubular ducts on the head (41.1) and labial setae (43.1-2)

The cladistic analysis shows the scale insects are a natural group The monophyly of the Coccoidea is supported by the following four autapomorphies of adult females and nymphs the presence of locular pores (1 1), the absence of wings (2 1) the occurrence of indistinct dorsal body tagmosis (3 1) and the presence of a single clow on the legs (31 1) However, the relationships of several family-group taxa, as well as the monophyly of some families remain controversial and not well established Further studies will be needed, with more taxa and particularly more anatomical or morphological characters, before the relationships are well understood

EVOLUTIONARY SCENARIOS

Two major attributes being studied here (habitats and protective structures) were then mapped onto the cladogram From this it was possible to propose an hypothesis of the ancestral states and then their evolution during the subsequent diversification of the superfamily Since the scale insects are sedentary (a few groups are weakly mobile), the term habitat here refers to the final settling and feeding site on the host plant There are difficulties in deriving evolutionary scenarios to explain the evolution of the habitats and protective structures for the entire family-level taxa of Coccoidea In large groups, such as the Margarodidae, Diaspididae, Coccidae and Pseudococcidae, position on the plant is highly variable in many lower taxonomic groups and, similarly, many taxa within a family have quite different types of protection For example, the Margarodidae *s.l.* constitutes a large heterogeneous group with considerable behavioral diversity, so that we can find scale insects in galls, on roots, on or under bark, or on twigs or leaves, depending on genus or species or even instar For these reasons, it is suggested that future research on the evolutionary scenarios of scale insects should concentrate on diversification within families

For the "habitat evolution" five attribute-states were used 1 bark, 2 branches, 3 roots, 4 leaves, 5 stem of grasses In the most parsimonious optimization, the cladogram (Fig 24) shows that the ancestral habitat attribute (feeding site) is equivocal and currently we can not choose between the aerial (bark) or underground (roots) habitats However, the bark or roots appears the most probable ancestral state since it requires only 8 changes against 9 for branches This scenario of other habitats being subsequent derivations implies 10 changes for leaves and stem of grasses *Stigmacoccus* lives on the bark of *Inga* spp *Matsucoccus*, is restricted exclusively to the bark and branches of *Pinus* spp Root feeding (*Margarodes*) has been adopted on a large diversity of plants, and is particularly characteristic of the cyst-forming Margarodinae This unique life-style may have evolved from that of leaf litter-inhabiting species which gradually moved underground and undoubtedly represents a secondary adaptation *Carayonema* is close to the supposed ancestral state, living on the superficial roots and having well developed legs, in contrast to *Margarodes* which live on deeper underground roots and have highly modified first

Source MNHN Paris

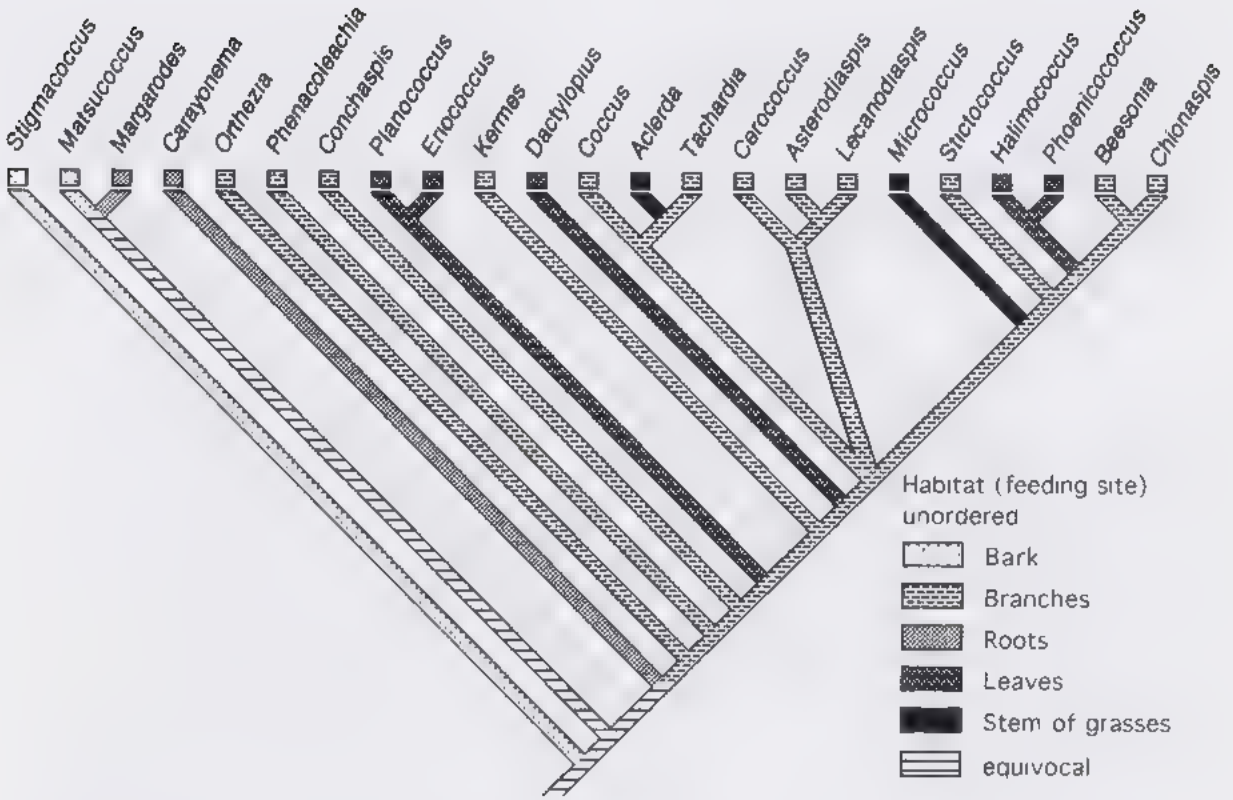


FIG 24 — Hypothetical evolution of the feeding site (habitat) in the Coccoidea, based on the phylogenetic tree in Fig 22

legs which are enlarged as digging organs The leaves habitat has arisen twice independently in *Phoenicococcus* + *Halimococcus* and *Planococcus* + *Eriococcus* + *Dactylopius* An importantchange has arisen in the clades of *Micrococcus* and *Aclerda* with a shift from woody hosts to grass host plants Such a shift, however, was not particularly successful as no wide adaptive radiation can be observed in these clades The branches habitat represents the most exploited feeding site among the Coccoidea, especially by the most speciose groups such as the Coccidae and Diaspididae.

For "protective structures", six states, according to the material used for protection, were identified 1 filamentous secretions, 2 amorphous secretions, 3 cyst (anal fluid), 4 test (formed by one or two types of secretions *e g* amorphous or filamentous secretion or both) 5 composed test (formed by filamentous secretions and anal fluid) 6 associated test (formed by filamentous secretions + anal fluid + exuvium) (Fig 25) The type of protection is strongly associated with habitat and a change in the protective method is correlated with a major change in way of life Thus, the adaptation of Margarodinae for underground life is accompanied by the use of anal fluid in the nymphal instars, whereas Margarodid species adapted to live under bark during the nymphal instars secrete amorphous substances In both cases, the adult females are mobile while

Source MNHN Paris

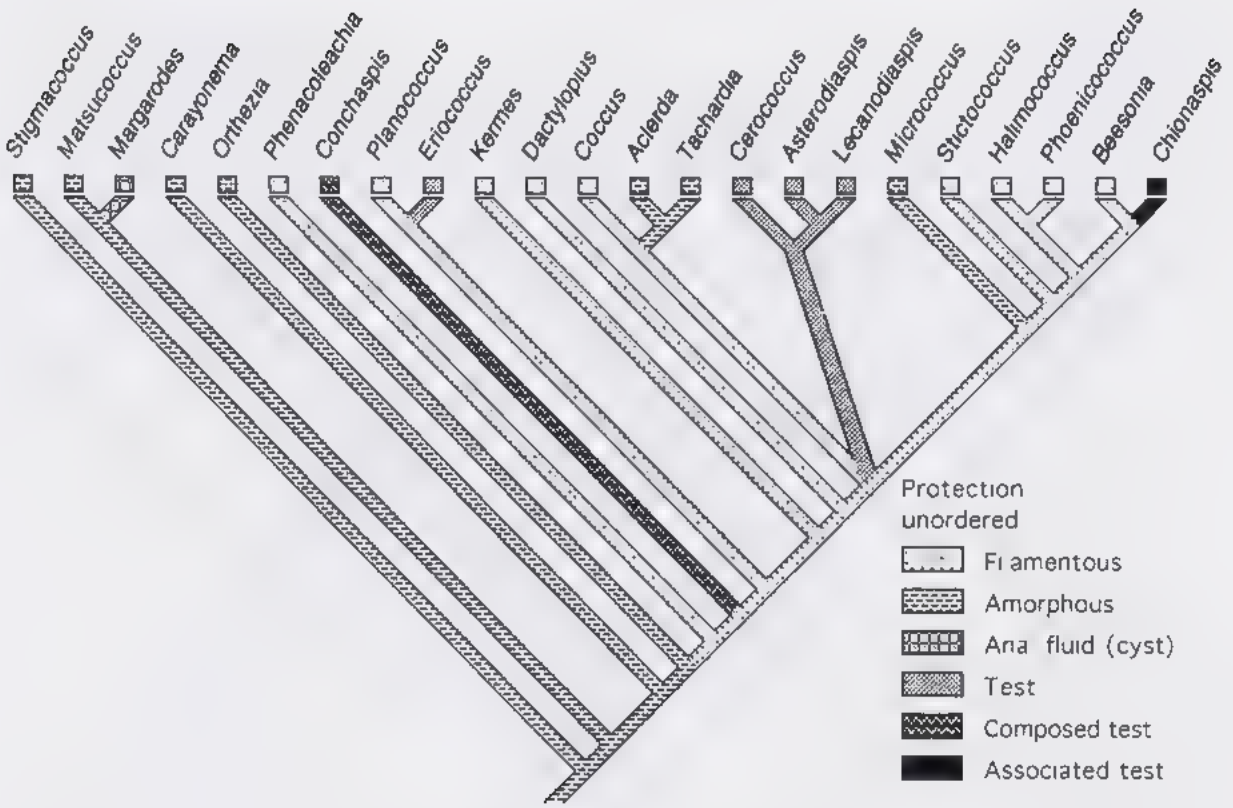


Fig 25 Hypothetical evolution of the protective structures in the Coccoidea, based on the phylogenetic tree in Fig 22

searching for males, but after mating produce filamentous secretions In cases for which the material used for protection varies in different stages of development, then the attribute state considered was that of the nymphal stage The most parsimonious scenario for the protective structures suggests that the ancestor was protected by amorphous secretions since it requires only 8 changes against 9 for filamentous secretions In *Stigmacoccus*, *Matsucoccus* and *Carayonema* spp , these amorphous secretions are secreted by cicatrice-like structures, while in the Ortheziidae, in which they evolved independently, they are secreted by the spine-like setae This scenario implies 10 changes for anal fluid and for various tests It is interesting to note that the use of anal fluids for constructing protective structures is found both in the basal clade Margarodidae then Conchaspididae and in the Diaspididae In general, for the group constituting mainly the lecanoid and diaspidoid taxa, it appears that the filamentous secretions were the ancestral state, with three separate lines evolving from it a felted sac (Eriococcidae), secreted tests (Lecanodiaspididae) and the composed test (Conchaspididae, Diaspididae) Under phylogenetic inference, this scenario for the evolution of protection suggests that lineage diversification was achieved with 9 changes within the superfamily This number obviously is related to the size of the data set Filamentous secretions represent the material most commonly used for protection These secretions have evolved independently in all clades of Coccoidea and are involved not only in protecting the body but also are vital to the functions of the respiratory, reproductive and excretory systems

Source MNHN Paris

The validity of these proposed evolutionary scenarios depends on several factors firstly, the validity of the proposed phylogeny, which was developed using heuristic methods and, therefore, is clearly approximate, secondly, the assumption that the families studied are monophyletic, which should be controlled in a more detailed cladistic analysis, and thirdly, the acceptance of the selected genera represent the characteristic of the families GULLAN (personal communication) would add further factors like the robustness of the data matrix, as the addition of further characters or taxa may alter tree topology, and the subjective nature of scoring of the character states (homologies may not be correctly recognized) Of course, the corroboration or refutation of these results is dependent on further testing with new data

DISCUSSION

One of the major evolutionary events in scale insect history led to adult females being neotenic and wingless, thus causing them to take on a sessile trophic habit, exploiting various microhabitats on the host plants while sucking sap from their host's vascular tissues This sedentary habit ensures that scale insects have a continuous, reliable food supply for minimal energy expenditure but with a major disadvantage that it can only be successful if the host-plant remains healthy In addition, being sessile makes them vulnerable to adverse environmental conditions and attack by natural enemies, so they have evolved a range of strategies to improve their chances of survival and reproductive success The manner of protection may vary as a function of the developmental stage, as in most species living in protected habitats, or may be constant throughout development, as in most species living in aerial habitats

It is obvious that optimal defensive strategies are those which give the greatest reduction in mortality and the greatest increase in reproductive success with the least energetic cost One of the most widespread of these defense strategies is the use of a series of wax secretions produced by an extensively developed wax gland system, even though this involves a considerable energy investment Obviously, the resources directed to secretions could not be allocated to oocyte production for the direct enhancement reproductive success The use of anal fluids represented progress in the energetic dispensation of resources because their use is particularly economic Indeed, using their own excreta, these insects would appear to invest very little energy in the production of the protective covers (cysts, composed tests, associated tests) compared with those which secrete wax from dermal wax glands to manufacture their protective covers Another ingenious method of protection for the scale insects, characterizing numerous unrelated taxa, consisted of behavioral adaptations that allow exploitation of the protection offered by the host plant (such as cryptic life beneath the bark, under leafsheaths or nodes of grass stem or gall formation), which lead to the reduction of the wax gland system The exploited host plant affords protection requiring less energy expenditure The cyst-forming Margarodinae found an alternative strategy to escape adverse environmental conditions by changing their generation time, as exemplified by the well known *Margarodes vitis* which can remain dormant but alive for up to seventeen years Apparently, simplest protection evolved in several unrelated groups by the modification of the female's own body (*e.g.* the marsupium of some Margarodids or the strongly sclerotized dorsum of many soft scales, Kermesids or Stictococcids) which functions in the same way as a secreted protective cover

What are the most successful scale insects and what is their defensive strategy ? These are the Diaspidids – small legless insects – which mostly live on the branches and leaves They

Source MNHN, Paris

construct, with an energetic compromise (*e.g.* using a mixture of secretions and anal fluids), the most elaborate test among the Coccoidea, which is formed throughout their life cycle, serving to protect the insect from the time of the 1st-instar nymph through to the ovipositing adult The efficiency of this protective cover is reflected by the success of the group since it includes more than 2000 species distributed worldwide In the case of the soft scales (the Coccidae), which have nearly 2000 species and tend towards a large body size, a series of alternative strategies has evolved, some using various and (sometimes considerable) secreted substances, others using plant or body protection The mealybugs, also have nearly 2000 species, are weakly mobile, and produce various waxy secretions (but these are much less important than those in the soft scales) and also occasionally use plant protection In the Margarodidae, species such as those in the genus *Matsucoccus* (Margarodidae *s.l.*) appear to represent the earliest known scale insects (fossils) but, at the same time, appear similar to the *Matsucoccus* spp of today, it is likely they were protected in a similar manner, *e.g.* by secreted amorphous and filamentous secretions

Most successful groups are adapted to the aerial part of woody host plants, particularly on the branches, which are the most exploited feeding sites by Coccoidea and associated with divers system of defense The protection in relation with this habitat (branches) shows an evolution from simple to complex structures branches + amorphous secretions, branches + filamentous secretions, branches + test, branches + composed test and branches + associated test The filamentous secretions, principally extruded by the pores and ducts, represent the material most utilized for protection Each taxon has found its own solution in ensuring their evolutionary success It may be significant that the groups which exhibit different types of protection during their life-cycle are characterized by a low species richness.

There appears to be an obvious coadaptation between body size, habitat selection and defense and this has caused convergent evolution of protection strategies dictated by habitat-required defense strategies A trend is observed in the defense strategies of scale insects to find a compromise between energy usage and the efficiency of the protective structures Understanding of the extant habitats and protection and how they could have evolved in scale insect history necessitates observation of their early evolution Based on paleontological data, the earliest known scale insects belong to the Margarodid genus *Matsucoccus*, from the Lower Cretaceous, living on *Pinus* spp , and the fossil Coccoids from Tertiary amber, within which all the main lineages of current families within Coccoidea have been found, are mainly Margarodids According to SHCHERBAKOV (1990), ancestors of scale insects were aphid-like four winged Precoccids but the current evolutionary hypotheses regarding the early evolution of Coccoids suggests that they lived in leaf litter WIGGLESWORTH (1972), in his book "The life of insects" suggested that the insects may have evolved in the litter layer KOTEJA (1984, 1985, 1990a,b) has also postulated that Coccoids may have evolved in the forest litter probably during the Permian to Jurassic, where they acquired adaptations to either an epigeic (all main lineages of Coccoidea) or an hypogeic (Margarodinae) life behavior This adaptive radiation of early Coccoids may have allowed them to colonize most parts of their host plants and it is a reasonable assumption that they displayed, at this period, most of their present morphological characteristics This is what we observe with the Margarodidae *s.l.* They exploit more feeding sites (roots (Margarodinae), bark and branches (Matsucoccinae, Steingeliinae), under bark (Xylococcinae), leaves (*Stomacoccus platani)* and galls (*Araucaricoccus* spp , *Matsucoccus gallicus)* than the Necoccoids, which are more specialized A probable secondary radiation started in the

Source MNHN, Paris

Cretaceous, with the colonization of the gymnosperms and angiosperms, mainly involving the recent Coccoids Most of the Neococcid fossils that originate from the upper Cretaceous are similar to contemporary forms which have evolved independently in various directions, colonizing all parts of their host plants KOTEJA (1985) suggested also since primary habitat was leaf litter, the scale insects may have fed on the dead and decaying remains of plants, particularly on fungi and bacteria that are responsible for plant decay Although the mouthparts of scale insects are adapted for piercing and sucking nutritious fluids from plants, it is possible to admit that they have could used decayed material as food in their early evolution Recently, *Carayonema orousseti* (Carayonemidae), was collected in the leaf litter in South America but actually it fed on roots located near the soil surface (J OROUSSET, personal communication) Another Coccoid, *Laurencella marikana* (Margarodidae) was found in a similar situation A colony of these Margarodids had settled on the parts of the roots located at the soil surface, covered by the leaf litter and stones (FOLDI, 1995b) We can suppose that similar situation may have occurred in the early evolution of Coccoids, where some aerial part of the roots were available for a transit to a new feeding site or habitat

To provide a fuller explanation for the adaptive evolution (habitat and protection) of scale insects, we need to carry out a detailed phylogenetic analysis on the Margarodidae *s.l.* since several fossils show that some of them had already reached their extant morphological organization early in Coccoid evolution

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Source: MNHN, Paris

What Did the Ancestors of the Woodroach *Cryptocercus* Look Like? A Phylogenetic Study of the Origin of Subsociality in the Subfamily Polyphaginae (Dictyoptera, Blattaria)

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ABSTRACT

Studies of relationships between *Cryptocercus* and termites have been biased because of the use of the misleading concept of "primitive taxon". Using the phylogeny of the subfamily Polyphaginae (including *Cryptocercus*), the traits ancestral to *Cryptocercus* and its sister-genus have been inferred. *Cryptocercus* appeared from an ancestor distributed in tropical forests of Indo-Asia, inhabiting treeholes or holes in termite nests, being gregarious and displaying an alarm behavior involving disruptive coloration of wings and pleural gland. The pattern of change from ancestral gregariousness toward derived subsociality in the case of *Cryptocercus* provides indications *by analogy* for a modification of the theories of social evolution in termites.

RESUMÉ

A quoi ressemblaient les ancêtres de la blatte xylicole *Cryptocercus*? Une etude phylogenetique de l'origine de la subsocialité dans la sous-famille des Polyphaginae (Dictyoptera, Blattaria)

Les recherches entreprises à propos des relations entre la blatte *Cryptocercus* et les termites ont été longtemps biaisées à cause de l'utilisation du concept erroné du « taxon primitif ». Avec l'analyse phylogenetique de la sous-famille des Polyphaginae (incluant *Cryptocercus*), il est possible de retracer les caractéristiques ancestrales à *Cryptocercus* et à son genre-frère. *Cryptocercus* s'est diversifié à partir d'un ancêtre vivant dans les forêts tropicales d'Inde et d'Asie qui habitait les troncs creux ou les termitières creusées, était grégaire et montrait un comportement d'alarme mettant en jeu une coloration disruptive et une glande pleurale. L'hypothèse d'un passage d'un grégarisme ancestral à la subsocialité dérivée dans le cas de *Cryptocercus* permet de proposer *par analogie* des modifications aux théories sur l'évolution de la socialité chez les termites.

INTRODUCTION

An understanding of evolutionary processes requires a search for both ancestral patterns and the way these patterns have changed in the course of evolution. This quest for ancestral patterns used either characters or taxa. In the past, taxa which were assessed as exhibiting some

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Source: MNHN, Paris

ancestral characters were misleadingly considered as representing wholly ancestral taxa and were thus named primitive taxa, missing links, living ancestors, forerunners, lower taxa, or stem-groups (*e.g.* ELDREDGE, 1987) These taxa were considered as such probably because it seemed intuitively more realistic and more simple from a gradist perspective to use some living taxa as ancestors than to analyze independently the evolution of many different characters Evolution was often simply traced between two extant taxa, from a so-called "ancestral taxon" to a so-called "evolved taxon", as if ancestor-descendant relationships could be inferred among present day terminals There are many examples of such kind of statements concerning cockroaches which were misleadingly considered as "primitive" or "ancestral" relative to termites (*e.g.* TILLYARD, 1936; RAU, 1941; WILSON, 1971, 1975)

This way of thinking is especially misleading because it implies, groundlessly, that most characters are primitive in a taxon by correlation with the primitive state of only a few traits under study (DAWKINS, 1987) It precludes any further advances or at least leads to unclear views, in the understanding of evolutionary processes (KUKUK, 1994) It is moreover phylogenetically nonsensical because phylogenetic characters must be considered *a priori independent* of one another and may be assessed *a posteriori* only *relatively* primitive, according to the principle of heterobathmy (HENNIG, 1966) Ancestors can never be reconstituted in their whole and we can only infer their plesiomorphies using optimisation on phylogenetic trees Their own autapomorphies have disappeared with them during their evolution Therefore, ancestors cannot be phylogenetically defined and they will remain paraphyletic taxa (ELDREDGE & CRACRAFT, 1980; NELSON, 1970, 1989)

It is such a paraphyletic picture that I intend to reconstitute here, when dealing with the ancestor of *Cryptocercus*. It should be carefully kept in mind that this picture does not represent any real living or extinct organism but is a hypothesis as to the character states that existed in an ancestor together with autapomorphic and forever unknown traits The prime interest of such a paraphyletic ancestral picture is to provide heuristic indications as to the evolutionary paths which have led to the present day situation in extant taxa The woodroach *Cryptocercus* (Fig 1a) has, since the study of CLEVELAND *et al.* (1934), been especially considered a "primitive taxon" or a "missing link" because it shows traits hypothesized as ancestral to termites, especially xylophagy and protozoan symbionts although the actually ancestral origin of these traits remains controversial (THORNE, 1990, 1991, NALEPA, 1991) This opinion has been discarded by GRANDCOLAS & DELEPORTE (1992, 1996) and GRANDCOLAS (1994a, 1994b, 1995a, 1996a) on the basis of the phylogenetic position of *Cryptocercus* This position has, hitherto, not been evaluated using modern phylogenetic methodology, and both a reappraisal of and search for characters and their cladistic treatment were obviously needed (DELEPORTE, 1988, GRANDCOLAS, 1994a, 1996a, GADE *et al.*, 1997) *Cryptocercus* cannot be a "primitive taxon"

FIG 1 — Some Polyphagmae and their habitats **a**: *Cryptocercus punctulatus* female with two young nymphs in the background, in their wood chamber **b**: *Therea petiveriana* female (bearing an ootheca) **c**: *Ergaula capensis* male and female (bearing an ootheca) **d**: treehole (*Desbordesia glaucescens*) with a termite nest sheltering *E capensis* in Gabon **e**: *Heterogamisca chopardi* female (bearing an ootheca) **f**: cushion shrub of *Salsola* sp beneath which *H chopardi* burrows, in Saudi Arabia

Source MNHN Paris

Source MNHN Paris

because "primitive taxa" cannot be identified, and, according to its phylogenetic position within the Polyphaginae (Fig 2), its xylophagy and its intestinal symbiosis must be assumed convergent with those of the so-called "lower termites" (GRANDCOLAS, 1995a, 1996a, GRANDCOLAS & DELEPORTE, 1992, 1996) *Cryptocercus* remains however a useful model for understanding and predicting by analogy what could have been the first stages of sociality in termites, relatively to xylophagy and protozoa symbiosis, if one is convinced that these latter traits are ancestral to termites and have determined their evolution toward eusociality (*e.g* MYLES, 1988, THORNE, 1990, ROISIN, 1994) By the way, it should be kept in mind that this latter hypothesis has not been tested by termite phylogenetic analyses

Cryptocercus as a member of the subfamily Polyphaginae (Fig 1) is also an interesting model to study the origin and evolution of a complex subsocial behavior in Insects *Cryptocercus* defends a wood chamber, feeds its nymphs and transfers to them protozoan symbionts via proctodeal trophallaxis In this paper, I examine the ancestral states of morphological, anatomical and behavioral traits of *Cryptocercus* involved in its subsocial behavior and in its potentially related behaviors such as habitat use and anti-predator behavior These states are inferred in reference to the best supported phylogenetic hypotheses concerning *Cryptocercus* and its relatives (Fig. 2)

MATERIAL AND METHODS

Phylogenetic reference Ancestral states for Polyphaginae and the corresponding derived states in *Cryptocercus* are inferred according to current phylogenetic reconstructions (GRANDCOLAS & DELEPORTE, 1992 GRANDCOLAS 1994a 1996a, Cockroach phylogenies are reviewed by GRANDCOLAS (1997a) GRANDCOLAS (1994a) presented a tree of the subfamily Polyphaginae (16 taxa, 50 characters CI = 0 79 , RI = 0 87) which is used here Phylogenetic analyses based on RNA and DNA sequences (VAWTER 1991, KAMBHAMPATI, 1995 1996) are not taken into account, because they used too small sequenced portions of respectively only 2 and 25 cockroach genera (belonging to a few subfamilies) and no or few genera of Polyphagidae except *Cryptocercus* Their results were internally inconsistent and moreover incongruent with each other and with both previous systematic concepts (PRINCIS, 1960 MCKITTRICK 1964) and later morpho-anatomical phylogenetic analyses (GRANDCOLAS, 1996a) KLASS (1995 presented a tree for Isoptera, Blattodea and Mantodea on the basis of the study of 14 species however, it was not constructed according to a genuine phylogenetic analysis (no matrix of characters, no outgroups, polymorphic characters not coded as such, etc) and cannot be taken into consideration According to all these considerations, GRANDCOLAS' (1994) tree is preferred to others for making evolutionary inferences because it has been obtained according to a much more extensive range of taxa and characters, and has a much higher consistency Moreover, it has received support from the analysis of hypertrehalosaemic neuropeptides from corpora cardiaca (GADE *et al* 1997) This does not preclude re-examination of results discussed here in the framework of a total evidence approach (KLUGE, 1989)

Attribute optimization on the tree None of the traits considered in this study were used in tree construction, except when mentioned Only those supported by primary homology (DE PINNA 1992) were used to build the tree (see GRANDCOLAS 1994, Extrinsic (*e.g* geographical distributions) or poorly defined (*e.g* social systems) traits are parsimoniously optimized on the tree (as unordered states using Wagner parsimony FARRIS, 1970), and are treated such as attributes (*sensu* MICKEVICH & WEBSTER 1990 GRANDCOLAS *et al* , 1994) This is in agreement with the principle of total evidence (KLUGE 1989), which should be applied only to primarily homologous traits (GRANDCOLAS *et al* 1994) The ancestral states of these different traits are commented upon here with respect to *Cryptocercus* if they are synapomorphic of [*Therea* + *Cryptocercus*] (the states immediately ancestral to the three described species of *Cryptocercus* are not mentioned as such)

Eight traits were selected Their states are listed below and in Figures 3-5 and 7-8 The states of these traits have been generalized for each genus on the basis of observations made on different species (Appendix 1) Geographical distributions were established according to taxonomic literature for all described species

Patterns of geographical distribution (Fig 3) The analysis of this trait was restricted to the clarification of the ancestral state of [*Ergaula* + *Eucorydia* + *Therea* + *Cryptocercus*] (GRANDCOLAS, 1994b) A simple optimization of the different distribution areas has been carried out on this sub-tree, without engaging in controversial methodologies of phylogenetic biogeography

Source MNHN, Paris

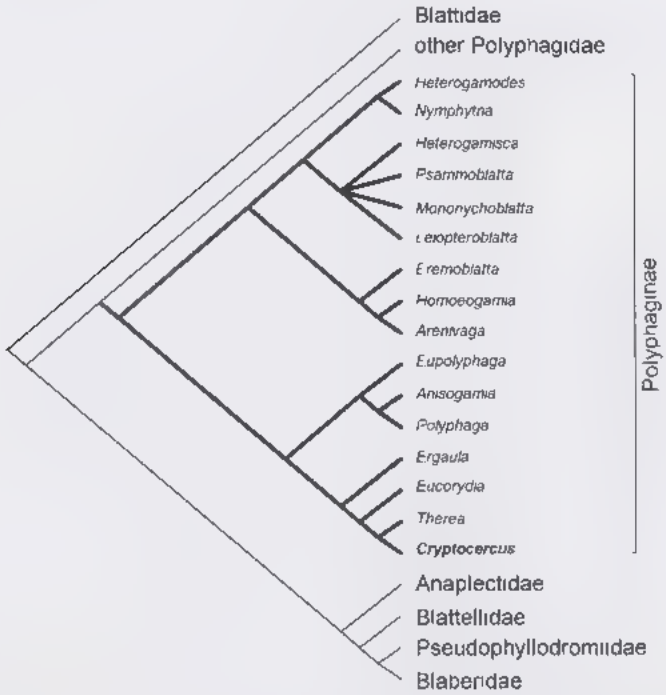

FIG 2 — A synthetic phylogenetic tree presenting the position of *Cryptocercus* relative to cockroach families, and nested in the subfamily Polyphaginae, according to the analyses of GRANDCOLAS (1994a, 1996a).

Biome occupancy (Fig 4) Five biomes or combinations of biomes are defined. "Temperate forest + desert" are considered as a single state and not as a polymorphism involving "temperate forest" and "desert" because the species displaying this state are distributed in all these biomes. Tropical forests include both rain and dry forests.

Habitat use (Fig 5) Six habitats are distinguished from ecological studies (CHOPARD, 1938, 1969, COHEN & COHEN, 1976, EDNEY *et al.*, 1974, GHABBOUR *et al.*, 1977, GRANDCOLAS 1994c, 1995a, 1995b, 1996b, 1997b, HAWKE & FARLEY, 1973, KAPLIN, 1996a, 1996b, LIVINGSTONE & RAMANI, 1978, NALEPA, 1984, 1988a, 1988b, ROTH & WILLIS, 1960, SEELINGER & SEELINGER, 1983, pers. obs.): "Caves or burrows" and "treeholes or termite nests" (Fig. 1f) are not polymorphic characters because some species inhabit both habitats depending on their relative availability. These habitats are combined as the same state because they are assumed to be similar and to indicate the choice of similar specific physical conditions, namely a cavity underground for "caves or burrows" or a cavity in a biotic structure for "treeholes or termite nests". Cockroaches inhabiting "sand beneath cushion shrubs" (*e.g.* Fig. 1d) do not burrow in "loose sand" and conversely.

Social behavior (Figs 6-7) Three different behaviors may be characterized, according to the classical definitions of MICHENER (1969) revised by EICKWORT (1981): solitary, gregarious and subsocial behaviors. In gregarious species (Fig. 6) larvae and/or adults aggregate independently of relatedness characteristics of genera according to the same studies as for habitat use). Subsocial species exhibit parental care for the larvae which remain close to their parents. In solitary species, cockroaches never aggregate, even when environmental conditions could force them to be close together. A discussion concerning these traits and their evolution in cockroaches may be found in GRANDCOLAS (1997c).

Anti-predator behavior (Fig 8) This behavior was coded using three different states: burrowing and freezing (in a loose substratum), disruptive alarm (using presumably disruptive coloration of fore wings with yellow spots and pleural glands, both traits used as characters in GRANDCOLAS, 1994a), and tremulation and obstruction (of galleries) (LIVINGSTONE & RAMANI, 1978; RITTER, 1964, SEELINGER & SEELINGER, 1983; FARINE & BROSSUT, pers. com., pers. obs.). During disruptive alarm behavior, adult cockroaches raise their wings and exert their pleural glands while larvae rapidly burrowed in the substratum. Wing coloration (Fig. 1b) and movements are assumed to disturb predators and to provoke confusion effects (perhaps not to warn them because gland products are not proved yet to be deterrent). Pleural glands are assumed to produce alarm pheromones and/or allomones (BROSSUT & SREANG, 1985). However, in the laboratory, pleural glands are also exerted

Source: MNHN, Paris

FIG. 3 — Distribution of the genera of the subfamily Polyphaginae

during conspecific encounters indicating that pleural glands may also assume other roles such as social or sexual communication (BROSSUT & FARINE, pers. com.). The disruptive alarm behavior may vary: some *Ergaula* species lack disruptive coloration on the wings but they have pleural glands and are able to exert them (*E. capensis* in Gabon, GRANDCOLAS, 1997b).

Ootheca laying behavior. The Polyphaginae either lay their oothecae within the substratum without gluing or coating, or they bury them (GHABBOUR *et al.*, 1977, GRANDCOLAS, 1994c, 1995a, 1996b, 1997b, pers. obs., KAPLIN, 1996b, MCKITTRICK, 1964, NALEPA, 1988a).

Diet and intestinal symbiosis. In addition these two attributes were taken into consideration but were not described in detail here because they have been studied previously (GRANDCOLAS, 1995a, GRANDCOLAS & DELEPORTE, 1996). Both have two states: xylophagous diet (in *Cryptocercus*) versus saprophagous diet and presence of flagellate intestinal symbionts (in *Cryptocercus*) versus absence.

Estimates of the derivative loads (BRINCK, 1977, ANDERSEN, 1979) were provided for each node inside the phylogenetic tree of the subfamily Polyphaginae to which *Cryptocercus* belongs. Derivative loads represent the number of derived characters in a taxon or at a node, relative to the total number of characters in the tree; these loads may be considered

Source: MNHN, Paris

estimates of the amount of evolutionary change that has occurred at each step in the diversification of a group. These derivative loads are estimated using the tree of GRANDCOLAS (1994a), with addition of autapomophic characters taken from other publications (GRANDCOLAS, 1993, 1994c) or from observations listed in Appendix 2. These estimates are obviously very imprecise since they are based on a relatively small sample of characters. They must not be considered as indicative of true evolutionary rates since they are free of clock assumptions (time periods are not assumed identical between nodes in cladistic trees).

RESULTS

Most parsimonious phylogenetic patterns

The optimization of traits on the phylogeny are shown in Figures 4-5 and 7-8. All equally parsimonious patterns are shown in the figures, the differences between these patterns do not influence the conclusions concerning the ancestor of *Cryptocercus*. According to these optimizations, 8 characters' states are listed for the ancestor of *Cryptocercus* (Table 1).

Distributional patterns (Fig. 3). Using a hypothesis of modification of an ancestral area by vicariance, [India + South Asia + East Asia] is inferred to be the ancestral area of the monophyletic group [*Ergaula, Therea, Eucorydia, Cryptocercus*], with secondary presumptive dispersals into tropical Africa (in the lineage leading to *Ergaula capensis*), and into North America (in the lineage leading to *Cryptocercus punctulatus*). Using the phylogenetic pattern, the ancestor of *Cryptocercus* is hypothesized to have been distributed in Indo-Asia.

Biome occupancy (Fig. 4). Two patterns implying four steps are equally parsimonious. They differ by changes in the group [*Eupolyphaga, Polyphaga, Anisogamia*]: either "temperate forest + desert" is ancestral to this group with a change to "desert" in *Anisogamia*, or "temperate forest + desert" appeared convergently in *Eupolyphaga* and *Polyphaga*. The subfamily originated in deserts and secondarily occupied tropical forests (ancestor of [*Ergaula + Therea + Eucorydia + Cryptocercus*]) and then invaded temperate forests (*Cryptocercus*). The ancestor of *Cryptocercus* was thus distributed in tropical forests.

TABLE 1. Characters' state of the ancestor of *Cryptocercus*, determined according to the optimizations on the phylogeny of the subfamily Polyphaginae. Optimizations of diet and intestinal symbiosis are given according to GRANDCOLAS (1995a) and GRANDCOLAS & DELEPORTE (1996).

Character	Ancestral state
Distribution	India + Asia
Biome	Tropical forests
Habitat	Treeholes or holes in termite nests
Social behavior	Gregariousness
Alarm behavior	Yellow spots on wings and pleural glands
Diet and intestinal symbiosis	Saprophagy and lack of intestinal Protozoa
Ootheca laying behavior	Without care

Habitat (Fig. 5). Two patterns implying eight steps are equally parsimonious. They involve the ancestral habitat "loose sand" for the subfamily, with either a change to "caves or

Source: MNHN, Paris

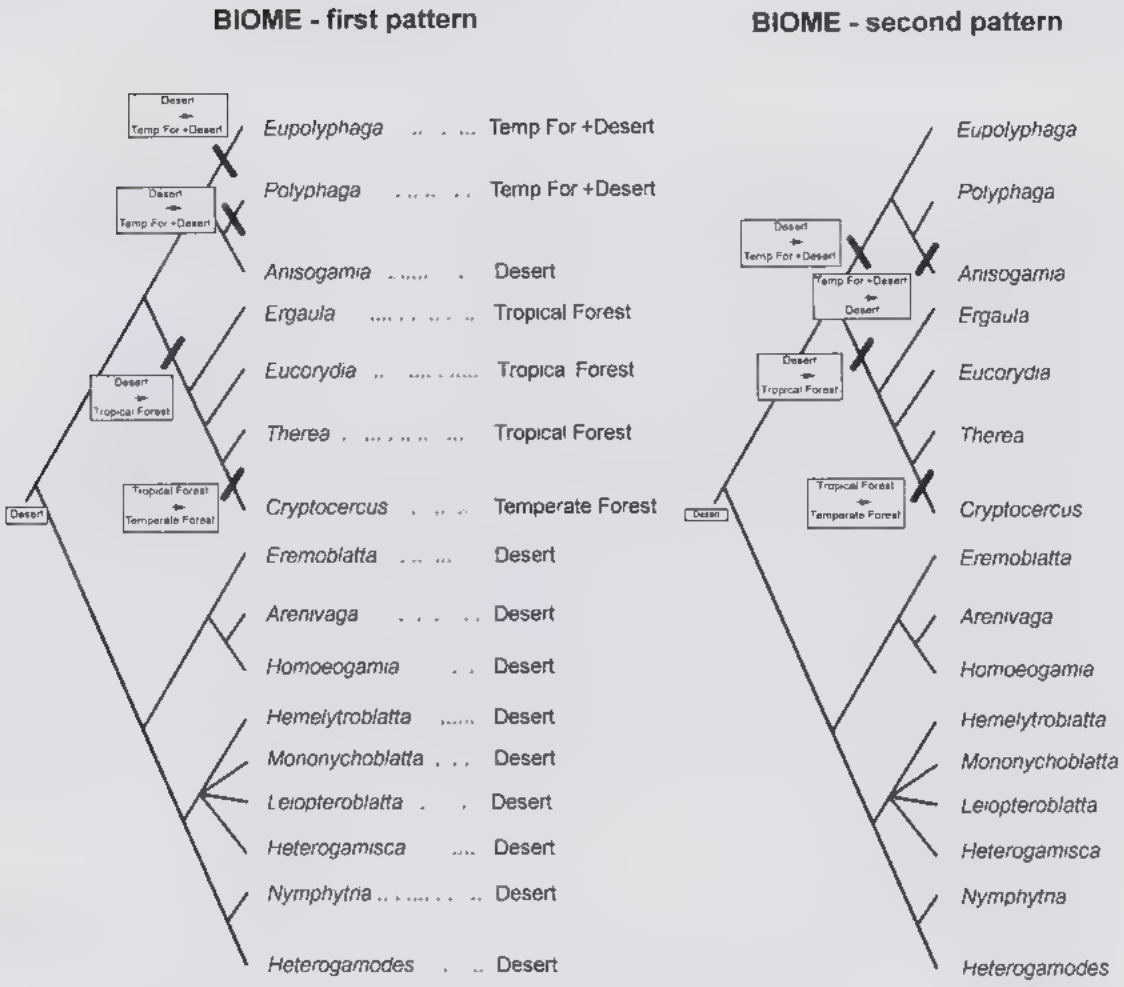

FIG 4 · Two most parsimonious patterns for the evolution of biome occupancy on the phylogenetic tree of the subfamily Polyphaginae The state changes are indicated on the branches where they take place

burrows" in the ancestor of [*Arenivaga* + *Homoeogamia*] with a subsequent reversal to "loose sand" in some species of *Arenivaga*, or two changes toward "caves or burrows" in *Homoeogamia* and some *Arenivaga* In either case, there was a shift toward "treeholes or termite nests" in the ancestor of [*Ergaula* + *Therea* + *Eucorydia* + *Cryptocercus*] Some species of *Therea* secondarily changed and inhabited "ground litter" and *Cryptocercus* switched to "rotten trunk" The ancestor of *Cryptocercus* inhabited cavities such as treeholes or termite nest holes

Social behavior (Fig 7) The ancestor of Polyphaginae was gregarious with two reversals toward solitariness occurred in species belonging to the genera *Heterogamisca* and

Source MNHN Paris

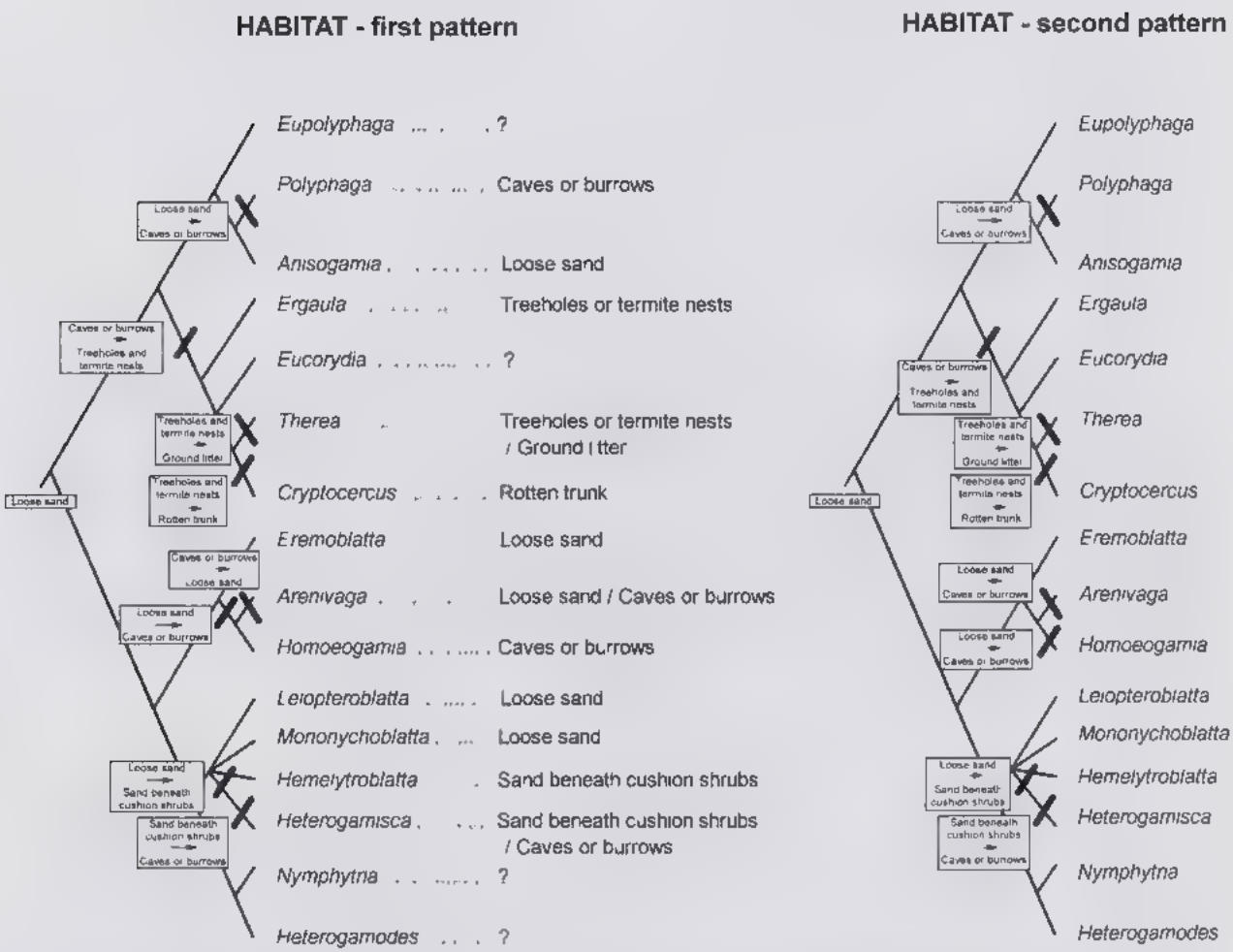

FIG 5 Two most parsimonious patterns for the evolution of habitat use, implying the habitat "loose sand" as an ancestral state Question marks indicate unknown states of attributes

Arenivaga Subsociality (familial brood care) appeared in *Cryptocercus*. Its ancestor was thus gregarious

Anti-predator behavior (Fig 8) According to the most parsimonious pattern, the ancestor of Polyphaginae showed "burrowing and freezing" as an anti-predator behavior Disruptive alarm is ancestral to [*Ergaula* + *Therea* + *Eucorydia* + *Cryptocercus*] Even though this state is partly inapplicable in the totally apterous *Cryptocercus* (for wing coloration), its ancestor may be inferred to have had disruptive alarm Pheromonal pleural glands and disruptive coloration of wings (Fig 1b) appeared in the ancestor of [*Ergaula* + *Therea* + *Eucorydia* + *Cryptocercus*] and disappeared in *Cryptocercus* which acquired a particular alarm behavior, combining tremulation in nymphs and defense of the chamber entrance by adults (gallery obstruction with pronotum)

Source MNHN Paris

Oothecal laying behavior All observed species of Polyphaginae deposit their oothecae in the substratum without any coating or gluing, except *Cryptocercus C. punctulatus* was observed to burrow a hole in dead wood, to deposit its ootheca inside and then to enclose it (McKittrick, 1964) The ancestor of *Cryptocercus* deposited its ootheca without care

Most of the changes occurring according to these parsimonious scenarios are combined to define an overall evolutionary scenario comprising several evolutionary paths (Fig 10) either gregarious ancestors in Polyphaginae have changed their biomes and remained gregarious or they have remained in the ancestral biome and changed their social behavior Gregarious ancestors living in a derived biome have also evolved toward subsociality.

Derivative loads

Autapomorphies of terminal taxa are listed in Appendix 2 Together with synapomorphies, there are 108 characters Loads are provided for each node of the cladogram (Fig 9) The most important loads for present discussion are present in the monophyletic group comprising *Ergaula, Eucorydia, Therea* and *Cryptocercus* The genera *Cryptocercus, Therea, Eucorydia* have especially high derivative loads, which are at least twice the mean value of other taxa (respectively 31 5 %, 24 1% and 25% relative to 9 5% as the mean) The difference between *Cryptocercus* and its common ancestor with *Therea* is also the highest value recorded at a node (12 1%) Clearly, among the set of characters examined, the amount of evolutionary change increases as one gets closer and closer to *Cryptocercus*

DISCUSSION

The ecological and behavioral ancestral attributes of Cryptocercus

Although it was never placed as the nearest relative of termites in any taxonomic or phylogenetic scheme (*e g.* HENNIG, 1981, THORNE & CARPENTER, 1992, see also KRISTENSEN, 1995), *Cryptocercus* has been considered to have a way of life ancestral to cockroaches and termites solely because of its xylophagy, protozoan symbionts and familial way of life (CLEVELAND *et al.*, 1934, GRASSÉ & NOIROT, 1959, WILSON, 1971, NALEPA, 1984, 1991, 1994, MYLES, 1988) Nonetheless, according to phylogenetic analysis (GRANDCOLAS, 1994a, 1996a) all these traits are actually apomorphic to *Cryptocercus* This does not support the hypothesis that *Cryptocercus* inherited these traits from a common ancestor with termites but supports the hypothesis of origin of these traits by convergence (xylophagy, social system) and transfer (symbionts)(GRANDCOLAS & DELEPORTE, 1992, 1996, GRANDCOLAS, 1994b, 1995a, 1996a) According to these statements, *Cryptocercus* and other subsocial cockroaches do not deserve thus to be compared with termites in a *strict* phylogenetic perspective (*contra* CRESPI, 1996)

Xylophagy, protozoan symbionts, and familial way of life were so firmly considered as ancestral to cockroaches and termites that their origin was never questioned Also, the origin of subsocial behavior was poorly investigated in insects using phylogenetic comparative biology because of the lack of phylogenies Using the phylogeny, it is possible to infer that the ancestor of *Cryptocercus* was distributed in tropical forests of Indo-Asia, and inhabited treeholes and/or holes in termite nests (Fig 10) It was gregarious larvae were clumped in the same cavity, often together with some adults which were not necessarily their parents These adults laid their oothecae without care in the loose litter at the bottom of cavities The ancestors of *Cryptocercus*

Source MNHN Paris

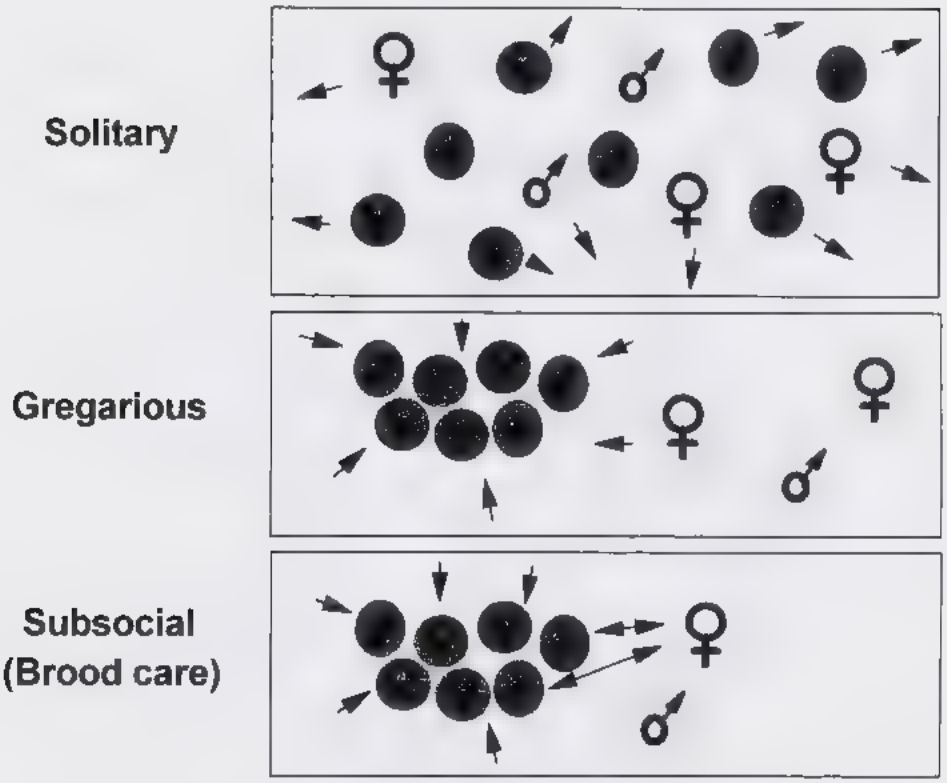

FIG 6 — A simplified representation of cockroach social systems involving adults (large and light grey circles) and larvae (small and dark grey circles), their interactions cohesive (arrows directed inward), or dispersive (arrows directed outward), and brood care (double arrows)

displayed a disruptive alarm behavior (raising fore wings with yellow spots and exerting pheromonal pleural glands) There were many important evolutionary changes from these ancestors since most of these character states were modified to account for the very different present aspect and behavior of *Cryptocercus* Derivative loads are especially high in the part of the cladogram close to *Cryptocercus* and increase sequentially at the dichotomies leading to [*Ergaula* + *Eucorydia* + *Therea* + *Cryptocercus*], [*Eucorydia* + *Therea* + *Cryptocercus*] and [*Therea* + *Cryptocercus*] Indeed, most of the characters sampled for phylogenetic studies change close to *Cryptocercus*, including its divergence from the common ancestor with its sister-group *Therea* From a gregarious ancestor, inhabiting cavities (*e.g.* Fig 1d) and showing a disruptive coloration, evolution produced a descendant which was subsocial, digging into the wood, lacking wings and strongly armored Unfortunately, most changes concerning biome, habitat, social system and alarm behavior occurred at the same node of the cladogram and it is thus impossible to assess the relative sequence of these different events using my phylogenetic hypothesis (Figs 4, 5, 7, 8) By analogy with the diversification of the subfamily Zetoborinae in South America, it is possible that the xylophagy of *Cryptocercus* appeared before it dispersed

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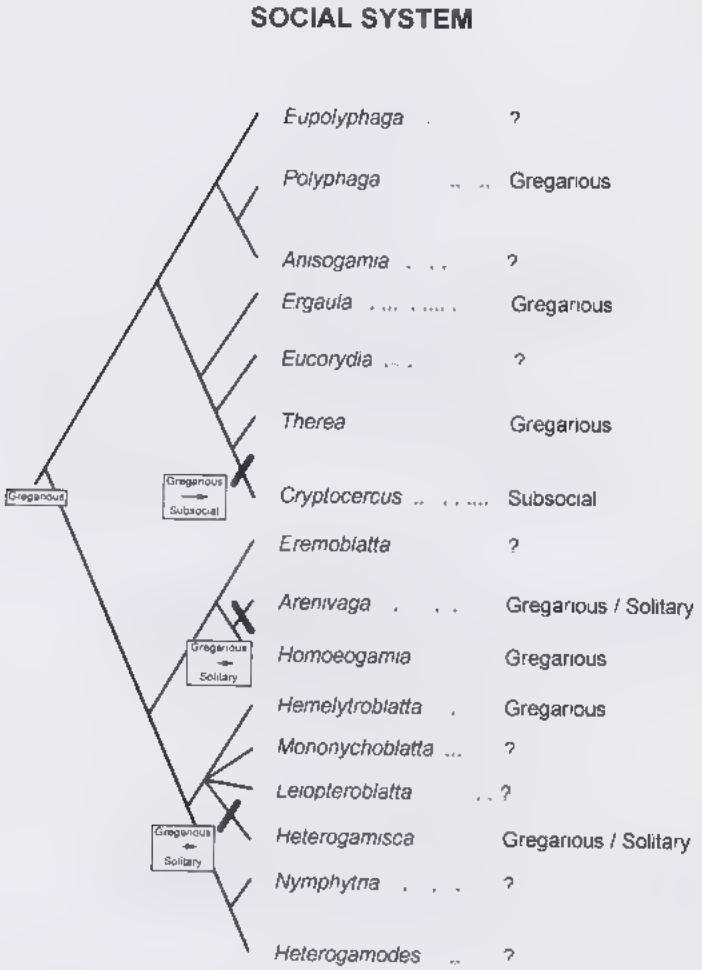

FIG 7 — Most parsimonious pattern for the evolution of social system.

to temperate forests because a wood diet and its life history correlates could facilitate adaptation to a seasonal temperate climate (see GRANDCOLAS, 1995a for an evolutionary scenario linking wood diet and adaptation to climate) It is necessary however to get additional data (*e.g* still unknown tropical *Cryptocercus* species or relatives) to substantiate this hypothesis in the present case of the subfamily Polyphaginae

The alarm behavior changed together with social system, but the different alarm behaviors displayed by gregarious and subsocial taxa require a high degree of behavioral coordination among conspecifics (so-called "cooperation" according to WILSON, 1975) This coordination could be related to behavior of cooperative groups (MILINSKI, 1979) They seem thus to be identical in this respect Alarm behavior appeared each time to cope with communication constraints imposed by each kind of habitat Species living in large cavities (Fig 1d)

Source MNHN Paris

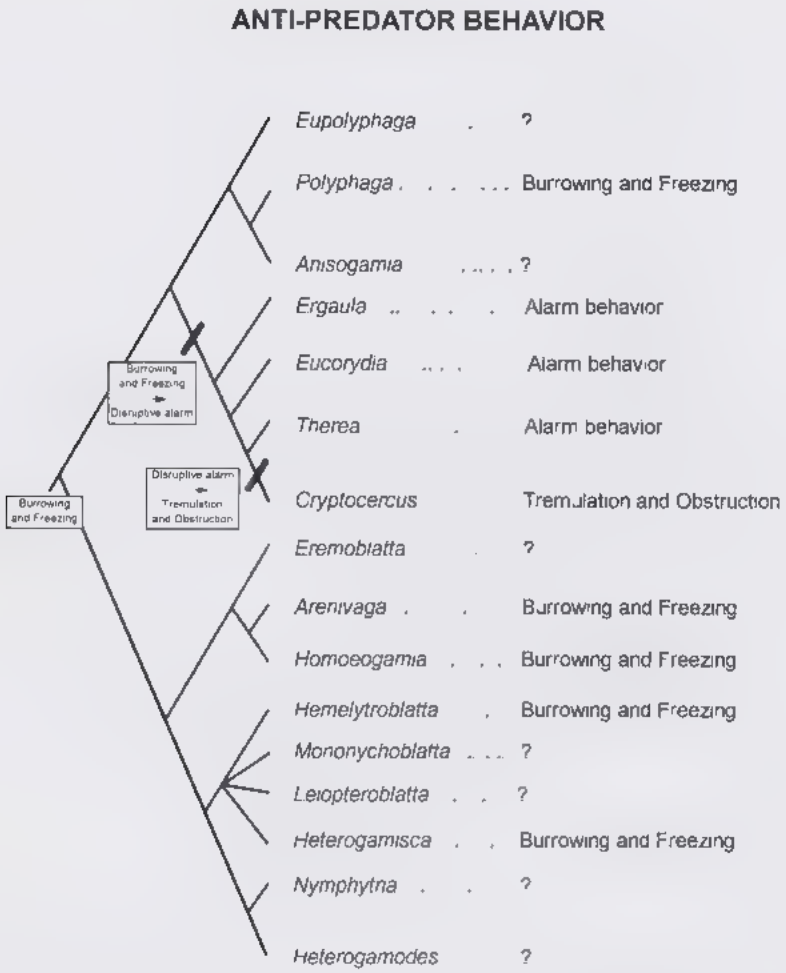

FIG 8 — Most parsimonious pattern for the evolution of anti-predator behavior

or in ground litter (the ancestor of *Cryptocercus* and *Therea* and *Ergaula* species) displayed a disruptive coloration (Fig 1b), an alarm or repugnatory gland and a burrowing ability This could protect them against large predators using visual perception (presumably vertebrates) Species living in small obscure chambers in rotten trunks displayed tremulation and gallery obstruction This could protect them against small predators detecting their prey with help of vibrations and odors (such as millipedes, spiders, etc , see MATSUMOTO, 1992 for depicting such predation events in similar cockroaches belonging to the subfamily Panesthiinae) Tremulation in an obscure cavity provides other members of the family with alarm larvae clump beneath the female who may close the gallery with her body (SEELINGER & SEELINGER, 1983)

Source MNHN Paris

From gregariousness to subsociality: an overlooked path

Most recent evolutionary theories regarded subsociality as a step toward eusociality Parental care (central to the concept of subsociality) is a standard principle of a kinship-based understanding of social evolution (*e g.* NALEPA, 1994, TALLAMY, 1986) Gregariousness and parasociality are often considered as more or less blind alleys, resulting from similar natural selection pressures to those exerted during the evolution of subsociality (*via* anti-predation, foraging, etc) but failing in this case to lead to more complex or integrated forms of sociality because of the lack of kinship between the members of the gregarious groups (WILSON, 1971, 1975)

In the subfamily Polyphaginae, a presocial behavior gregariousness clearly preceded the appearance of subsocial behavior in *Cryptocercus* (Fig 10) What could be the significance of such a pattern? Could presociality be exaptive (*i.e* preadaptive) for subsociality? This kind of prospect is a process-oriented question and deals with models of selection whereas phylogenies depict evolutionary pattern In this way, it could be hypothesized that both tolerance to conspecifics and behavioral coordination may be selected in a context of gregariousness and may be highly exaptive in a subsocial context Tolerance to crowding and interattraction are the first (pre-)requisites of social relationships (ALLEE *et al.*, 1949, GRASSE, 1952), as revisited recently by CRESPI (1994) Both tolerance and interattraction could be first acquired during the evolution of gregarious life In gregarious species, individuals cluster together because they are interattracted together Living in aggregations, they must tolerate spatial proximity with their conspecifics and do not spend time or waste energy in aggressive or dispersive behaviors Both tolerance and interattraction could be inherited in subsocial descendants where they could have an exaptive value because subsocial mother and larvae are closely associated and have mutual interactions which necessitate both behaviors The only difference between gregarious and subsocial species could be the propensity of individuals to show tolerance and attraction toward different kind of conspecifics, respectively in the context of non-kin individuals or in the context of a family In the same way, the alarm behavior of gregarious ancestors could be mediated by the reactivity to movements of non-kin conspecifics via specialized mechanical receptors This reactivity as well as these receptors could have been inherited by their subsocial descendants and have a high selective value when displayed with kin conspecifics during their own alarm behavior An efficient alarm behavior has, by itself, a high selective value because it allows individuals to escape death or injury by predators or parasites All the alarm behaviors described in the Polyphaginae were displayed through communication within some groups of individuals In the same way as for social system, the sensory and neurological basis for this communication may be selected first in one context (between non-kin) and then used secondarily in a somewhat different context (between kin) According to this possible exaptive value of gregariousness toward subsociality, a possible path for the appearance and change of social system could be traced from gregariousness to subsociality, and possibly to eusociality by *analogy* to termites This hypothesis of exaptive value of gregariousness toward subsociality is quite different from the statements that parental care seems to be more frequent in ovoviparous taxa or in oviparous taxa carrying oothecae a long time than in most oviparous GRANDCOLAS (1996) and NALEPA & BELL (1997) independently reported this trend respectively in reference to a phylogenetic hypothesis and to a traditional classification, respectively Ovoviviparity or long-carrying of

Source MNHN Paris

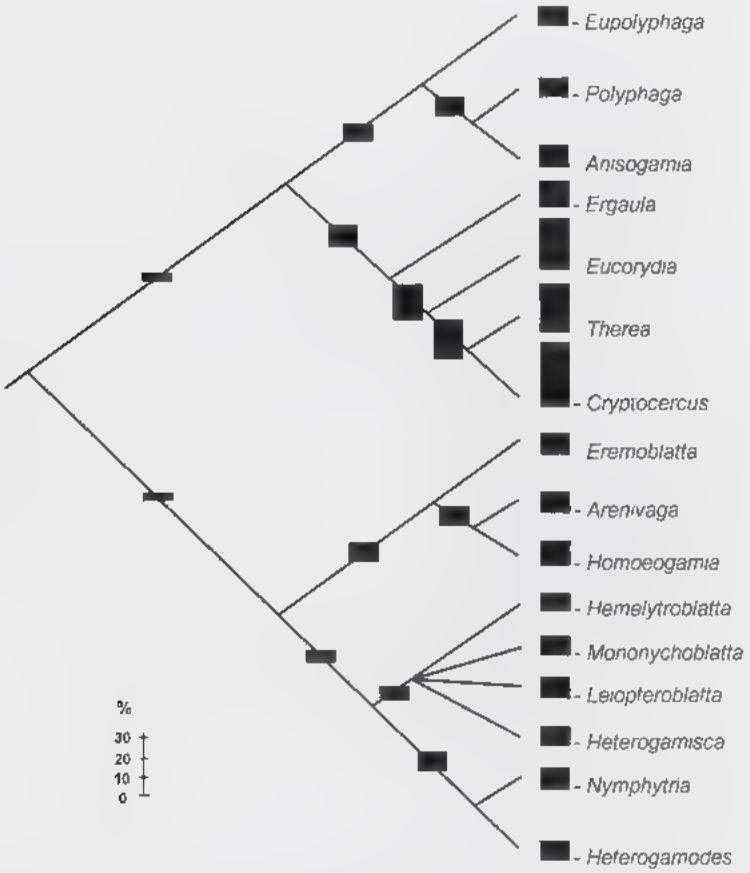

FIG 9 Derivative loads (ratios of apomorphies relative to the subfamily groundplan sampled with 108 characters) indicated on the phylogenetic tree of the subfamily Polyphaginae

oothecae are supposed by both papers – at least among several factors to increase and promote the relationships between the female and the larvae which are necessarily close following the brood birth These statements did not imply necessarily that gregariousness is ancestral and exaptive to parental care but merely that particular reproductive mode or more generally life history may promote parental care

Insights by analogy concerning the evolution of termites

Evolutionary inferences concerning the appearance of sociality in termites have always considered the prominent role of subsociality (parental care) in xylophagous ancestors Ancestors were hypothesized to be xylophagous and to harbor intestinal symbionts (CLEVELAND *et al*, 1934, GRASSÉ & NOIROT, 1959) This symbiosis would have determined a subsequent evolution toward parental care which is needed for transferring symbionts (*via* proctodeal trophallaxis between mother and larvae) This care for young nymphs could have shifted from adults to older nymphs and this could have been responsible for the emergence of a worker caste (NALEPA,

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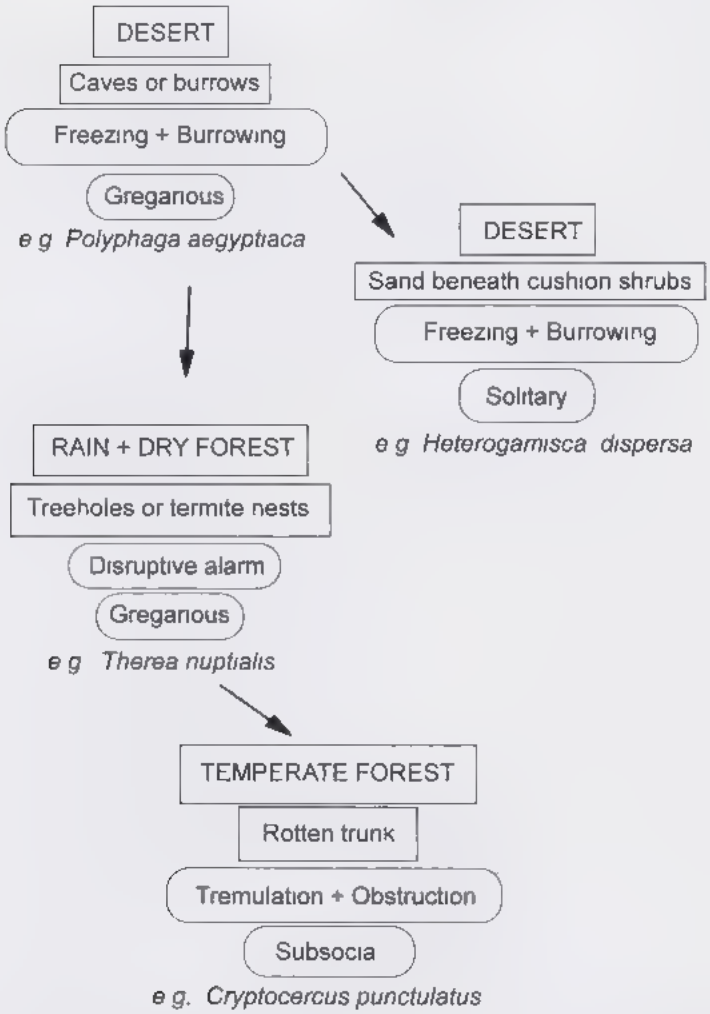

FIG 10 Several possible evolutionary paths according to the most parsimonious scenarios concerning biome, habitat and social behavior depicted in the Figures 3, 4 and 6

1988b, 1994) This scheme is, however, dependent on the assumptions that xylophagy and symbiosis were both ancestral to termites *Cryptocercus* is unfortunately not useful for testing these assumptions directly *by homology* because it is not closely related to termites (GRANDCOLAS & DELEPORTE, 1992, 1996) However, *by analogy*, the patterns in the clade to which *Cryptocercus* belongs suggest that an ancestor of termites could have exhibited a particular alarm or anti-predator behavior This behavior could have promoted an evolution toward subsociality as well as symbiosis and parental care This kind of evolutionary relationship between anti-predator behavior and sociality has already been postulated concerning insects (*e g.* STARR, 1985, CRESPI, 1994, but see KUKUK *et al.*, 1989) Unfortunately, chemical or other defenses of alates in termites are very poorly known (DELIGNE *et al.*, 1981, GRASSÉ, 1986) The studies of MOORE (1968, 1969) interestingly suggested that many termites (including alates of Mastotermitidae and Termitidae) have mandibular glands with a defensive role (quinone-secreting) a so widely distributed gland could be an indication of an ancestral pattern of

Source MNHN Paris

defensive behavior similar to that shown in cockroaches However, there is also few phylogenetic analyses of termites, except a limited molecular attempt (KAMBHAMPATI *et al.*, 1996) additional work is thus needed to understand whether a particular defensive behavior operated in the ancestor of termites

In conclusion, searching for patterns ancestral to the subsociality of *Cryptocercus* provides useful insights concerning the evolution of sociality First of all, the previous assumptions of relictual ancestral subsociality associated with xylophagy and intestinal symbiosis as shared ancestrally by *Cryptocercus* and termites are discarded The ancestors of *Cryptocercus* clearly lacked these traits Second, gregariousness, subsociality and anti-predator behavior are associated in the same phylogenetic pattern This pattern may be used to implement current models of social evolution which are too narrowly based on the relationship between parental care and resource use

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APPENDIX 1 — Species which are used for the generalizations at the generic level of the ecological and behavioral traits under study (information pertaining to each species may be found in the references cited in the material and methods section).

Arenivaga apacha (SAUSSURE, 1893)
A. bolliana (SAUSSURE, 1893)
A. erratica (REHN, 1903)
A. floridensis CAUDELL, 1918
A. investigata FRIAUF & EDNEY, 1969
A. tonkawa HEBARD, 1920
A. sp.
Cryptocercus punctulatus SCUDDER, 1862
C. relictus BEY-BIENKO, 1935
Ergaula capensis (SAUSSURE, 1893)
E. carunculigera (GERSTAECKER, 1861)
E sp.
Eremoblatta subdiaphana (SCUDDER, 1902)
Eucorydia dasytoides (SAUSSURE, 1864)
E. ornata (SAUSSURE, 1864)
E westwoodi (GERSTAECKER, 1861)
Hemelytroblatta (=*Psammoblatta*) *africana* (LINNAEUS, 1758)
Heterogamisca chopardi (UVAROV, 1936)
H. dispersa GRANDCOLAS, 1994
H marmorata (UVAROV, 1936)
Homoeogamia mexicana BURMEISTER, 1838
Leiopteroblatta monodi CHOPARD, 1969
Mononychoblatta semenovi CHOPARD, 1929
Polyphaga aegyptiaca (LINNAEUS, 1758)
P. indica WALKER, 1868
P. pellucida (REDTENBACHER, 1889)
P. saussurei (DOHRN, 1888)
Therea petiveriana (LINNAEUS, 1758)
T. nuptialis (GERSTAECKER, 1861)

Source MNHN Paris

APPENDIX 2 — Autapomorphies of different genera of the subfamily Polyphaginae, in addition to those listed in previous publications (GRANDCOLAS, 1993, 1994a, 1994c)

Genus	Autapomorphy
Anisogamia	pronotum impressed on the middle large mesonotum and metanotum (homoplastic with *Leiopteroblatta*) tarsal claws short
Arenivaga	antero-ventral margin of female fore femora with a long row of short and strong spines closely inserted supra-anal plate moderatley lengthened and emarginate
Cryptocercus	pronotal sculpture eyes reduced loss of setae on external sclerites abdominal segment VII expanded intestinal pouch (for flagellates) lack of clypeo-frontal suture wings totally lacking lack of cercal spheroid sensilla (homoplastic with *Eucorydia*) lack of pleural glands hook L3d of male genitalia not at all protruding ventral phallomere in male genitalia leg spines strong inter-tergal glands (FARINE *et al.*, 1989)
Eremoblatta	middle and hind femora without apical spine male subgenital plate very asymmetrical with two lateral projections
Ergaula	third frontal hollow females brachypterous while males macropterous (homoplastic with *Homeogamia*) fronto-clypeal suture invaginated where it joins the median suture
Eucorydia	metallic coloration of pronotum yellow spots on wings reaching the fore margin postclypeus flat hook L3d of male genitalia short lack of cercal spheroid sensilla (homoplastic with *Cryptocercus*) hind tubercle of R2 with a finger-like apophysis neoformation with a projected hind lobe fore tubercle of R2 projected
Eupolyphaga	setae with reddish coloration L3d and L3v enveloping and rounded outer outline of female eyes not rounded dorsally and ventrally
Hemelytroblatta	female subgenital plate with a median constriction
Heterogamisca	hind tibiae curved front flat alongside fronto-clypeal suture anal field of fore wings broad

Source: MNHN, Paris

APPENDIX 2. — continued

Heterogamodes	small tarsal claws (homoplastic with *Anisogamia*) female pronotum with strongly and regularly convex fore border large tergal glands just below the metanotal hind margin
Homoeogamia	female winged (homoplastic with *Ergaula*, *Eucorydia*, *Therea*) male fore wings with wide and horizontally subcostal field) male pronotum wider on the fore border female subgenital plate with two flaps
Leiopteroblatta	wings short with veins not visible male eyes small one tarsal claw (homoplastic with *Mononychoblatta*) hind femora spatulate and wide antero-ventral carena on middle and hind femora without spines but with long and strong setae small ocelli in males
Mononychoblatta	tarsae with only one claw (homoplastic with *Leiopteroblatta*) large metanotum and mesonotum (homoplastic with *Anisogamia*)
Nymphytria	small pronotum mesonotum laterally large hind femora spatulate only in the apical half lack of tarsal claws in females
Polyphaga	pronotum with white fore margin contrasting with dark coloration (homoplastic partly with *Hemelytroblatta*)
Therea	at least four yellow spots distinctly colored on fore wings fore wings black between yellow spots L2v diameter not increasing L3v posteriorly protruding Neoformation flattened

Source MNHN Paris

Early Evolution of the Lepidoptera + Trichoptera Lineage: Phylogeny and the Ecological Scenario

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ABSTRACT

New insights in the basal phylogeny of the Lepidoptera shed light on some topical issues in the debate over ecological aspects of the early evolution of this insect "order" and its sister group, the caddisflies. The currently best supported phylogeny of the basal lepidopteran clades is Micropterigidae + (Agathiphagidae + (Heterobathmiidae + (Eriocraniidae + (Acanthopteroctetidae + (Lophocoronidae + (Neopseustidae + (Exoporia + Heteroneura)))))))), *i.e.*, it is a richly branched "Hennigian comb". The larvae of Micropterigidae are "soil animals" which feed on foliose liverworts, fungus hyphae and decaying angiosperm material; they live in very moist habitats which probably differed little from those of ancestral Amphiesmenoptera. Exoporian larvae may similarly be broadly classified as "soil animals", and their ancestral life-style was probably very similar to that of micropterigid larvae, except that they lived in silken webbings/galleries. While the exoporian life-style might a priori be considered a retained plesiotypic trait, this interpretation is rejected because splitting events "basad from" the Exoporia + Heteroneura clade repeatedly led to canopy-living clades. It is most parsimonious to consider the ground dwelling of exoporian larvae to represent a secondary habitat shift. The crochet-bearing larval prolegs ascribed to the ground plan of Exoporia + Heteroneura apparently developed in response to a selective pressure for enhancing grips on a silken webbing, rather than for enhancing movement on a smooth plant surface (although the latter role may be the principal one in the bulk of the Lepidoptera). The preferred cladogram necessitates the assumption that an eclosion mode non-dependent on movable pupal mandibles evolved twice in the Lepidoptera: in the Lophocoronidae and in the Exoporia + Heteroneura clade. Larval invasion of genuine aquatic habitats is the key innovation of the trichopteran clade. Problems of recognizing an adult caddisfly as such are briefly discussed; they are particularly serious in the case of fossils. Contrary to the claim of one school of thought on ancestral caddisfly ecology, out group evidence from the Lepidoptera lends no support to a theory of ancestral caddisflies living in silken tubes. The basalmost lepidopteran clade whose larvae live in silken galleries (the Exoporia) did not arise until the eighth splitting event recognizable among extant forms.

RÉSUMÉ

Les premiers stades de l'évolution dans la lignée des Lepidoptères + Trichoptères : phylogénie et scénario évolutif

Une récente mise au point de la phylogénie basale des Lepidopteres permet d'inferer une nouvelle reconstitution des premiers stades de l'evolution de l'ecologie des Lepidoptères et de leur groupe-frere, les Trichoptères. La phylogenie des clades basaux de Lepidoptères la mieux corroboree actuellement est en fait un arbre hennigien « en peigne », arbre abondamment pourvu en rameaux : Micropterigidae + (Agathiphagidae + (Heterobathmiidae + (Eriocraniidae + (Acanthopteroctetidae + (Lophocoronidae + (Neopseustidae + (Exoporia + Heteroneura)))))). Les larves de Micropterigidae sont des « animaux du sol » qui se nourrissent d'hepatiques, d'hyphes de champignons, et de fragments d'Angiospermes en décomposition ; elles vivent dans des habitats tres humides qui different probablement très peu de ceux des Amphiesmenoptera ancestraux. Les larves d'Exoporiens peuvent être elles aussi considerées *grosso modo* comme des

N. P. KRISTENSEN, 1997. Early evolution of the Lepidoptera + Trichoptera lineage: phylogeny and the ecological scenario. *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects. Phylogenetic Tests of Evolutionary Scenarios. *Mém. Mus. natn. Hist. nat.*, **173**: 253-271. Paris. ISBN : 2-85653-508-9

Source : MNHN, Paris

« animaux du sol » et leur mode de vie ancestral etait probablement très semblable à celui des larves de Micropterigides, a l'exception du fait qu'elles vivaient dans des toiles/galeries de soie Le mode de vie exoporien pourrait être considere *a priori* comme la conservation d'un trait plesiotypique mais cette interpretation est rejetee parce que la cladogenèse des Exoporia + Heteroneura a conduit de maniere repetee à l'emergence de groupes vivant dans la canopee Il est donc plus parcimonieux de considerer la vie au niveau du sol des larves d'exoporiens comme une acquisition secondaire Les fausse-pattes larvaires munies de crochets, une caracteristique du plan de base des Exoporia + Heteroneura, se sont apparemment developpees en reponse a une pression de selection avec comme fonction l'agrippement sur une toile de soie, plutôt que le déplacement sur les surfaces lisses des vegetaux (bien que cette dernière fonction puisse être la plus courante chez les Lepidopteres). Le cladogramme retenu implique qu'un mode d'émergence independant de mandibules nymphales mobiles est apparu deux fois chez les Lepidopteres chez les Lophocoronidae et chez le clade des Exoporia + Heteroneura La conquête par les larves de veritables habitats aquatiques est l'innovation-cle du clade des Trichoptères Les problemes que soulève la caracterisation des Trichopteres adultes sont brievement discutes , ils sont particulierement importants dans le cas des fossiles Au contraire de ce qui etait affirmé par une ecole de pensee au sujet de l'ecologie des ancêtres de Trichoptères, la référence aux Lépidopteres en termes d'extra-groupe n'amène pas d'arguments en faveur de la vie dans des tubes de soie pour les ancêtres des Trichoptères Chez les Lepidoptères, le plus basal des clades ayant des larves vivant dans des tubes en soie (les Exoporiens) n'est pas apparu avant le huitieme événement de cladogenèse identifiable au sein des formes actuelles

INTRODUCTION

While many (indeed most, KRISTENSEN, 1995) current hypotheses about interrelationships of the higher insect taxa conventionally ranked as "orders" remain inadequately supported, there is very firm support for the monophyly of the entity Amphiesmenoptera, comprising the Trichoptera (caddisflies) and the Lepidoptera Numerous likely amphiesmenopteran groundplan autapomorphies have been identified in structural traits, and the entity consistently comes out as a monophylum in the molecular analyses I have seen (PASHLEY *et al* , 1993, WIEGMANN, 1994, REGIER *et al.*, 1995; WHEELER, unpublished).

The Amphiesmenoptera as a whole are one of the most species-rich lineages within the endopterygote insects, hence within the living world, and the Lepidoptera include the largest lineage of primarily herbivorous animals (POWELL *et al.*, in press) Considerable attention has been paid to the patterns of early phylogenetic diversification within the Amphiesmenoptera, and as far as the lepidopteran lineage is concerned a large basal section of the phylogenetic tree now appears fully resolved

In recent years the application of "tree thinking" to life-history traits has increasingly been taking evolutionary "scenarios" beyond the narrative stage, MILLER & WENZEL (1995), and references cited therein, provide a timely introduction to (entomological aspects of) this exciting field The present contribution briefly addresses some major questions concerning the ecological scenario of early lepidopteran evolution in the light of recently gained phylogenetic insights It also addresses some topical issues in the debate over early caddisfly evolution

THE ANCESTRAL AMPHIESMENOPTERAN

The numerous structural autapomorphies identified in the groundplan of the Amphiesmenoptera are reviewed elsewhere (KRISTENSEN, 1984b, KRISTENSEN & SKALSKI, in press, have a corrected/updated account)

The said apomorphies notwithstanding, the ancestral amphiesmenopteran must be characterized as an overall quite generalized endopterygote insect Thus it is notable that this ancestor, in the adult stage, must have retained very primitive traits in the mouth apparatus including, *e.g* , a movable labrum with extrinsic (frontal) retractors, mandibles with tentorial adductors, and a labium with distinct paraglossal lobes These plesiomorphies are still present in

Source MNHN, Paris

the basalmost extant Lepidoptera, and nowhere else among panorpoid endopterygotes The adult ancestral amphiesmenopteran may well have been a spore-/pollen-feeder like two of these basal lepidopteran families (Micropterigidae and Heterobathmiidae) In any case the moths in question (which do not together constitute a monophylum[1]) have in their preoral cavity some structural specializations (epipharyngeal brushes, spinose infrabuccal pouch) related to their feeding habits, and these specializations show remarkable similarities with those present in some of the (similarly pollen-feeding[1]) basalmost Hymenoptera (VILHELMSEN, 1996), which presumably are the closest amphiesmenopteran outgroups that take solid food as adults The suggestion seems straightforward that (as already suggested by MALYSHEV, 1968) adult spore/pollen-feeding was ancestral in a large monophylum comprising the Hymenoptera plus the panorpoid orders (KRISTENSEN, 1984b)

The larvae in the basal lineages of the other panorpoid insects (Mecoptera, Siphonaptera, Diptera) may be broadly characterized as "soil animals" (Fig 1) It is true that nannochoristid scorpionflies (presumably the sister group of all other Mecoptera, and overall generalized panorpoid insects) have aquatic larvae, but I am firmly of the opinion that this trait is a specialization *sui generis* in the family, rather than a retained plesiotypic condition the last-instar larva has open spiracles and the last (non-feeding) phase of this instar is spent in the soil outside the stream (PILGRIM, 1972) Similarly, I believe that those dipteran larvae which are aquatic are all secondarily so The outgroup criterion thus lends support to the notion that also the larvae of ancestral Amphiesmenoptera were soil dwelling, *i e* , that this life-style in extant Lepidoptera-Micropterigidae is genuinely plesiotypic

THE LEPIDOPTERAN LINEAGE

The groundplan autapomorphies of the Lepidoptera are reviewed in the references cited above for the Amphiesmenoptera Since lepidopteran larvae initially remained in what is believed to have been the environment of their amphiesmenopteran ancestors, it is unsurprising that their (few) groundplan autapomorphies include none one would immediately consider to be potential environmental adaptations Note, however, that contrary to a widespread belief the ancestral lepidopteran larva probably had a prognathous head, the structure of which may be somehow related to the life in narrow crevices in the soil/periphyton Prognathism itself is probably plesiomorphic at the basal amphiesmenopteran level (larvae of annulipalpian and "spicipalpian" caddisflies are prognathous, and so are those of Mecoptera-Nannochoristidae), hence at most an exaptation *sensu* GOULD & VRBA (1982) However, the elongation of the pleurostome (which is a specialization characteristic of derived prognathan heads) is a lepidopteran groundplan autapomorphy (KRISTENSEN, 1984a), the presence of this state in the otherwise typically hypognathous head of higher lepidopteran larvae is a morphological anomaly (DENIS & BITSCH, 1973), which apparently is best explicable in terms of phylogenetic constraints

An outline of basal lepidopteran clades

Principal recent references on the evolution of the basal lepidopteran lineages are KRISTENSEN (1984b, in press a, b), KRISTENSEN & SKALSKI (in press), DAVIS (1986, 1987),

Source MNHN, Paris

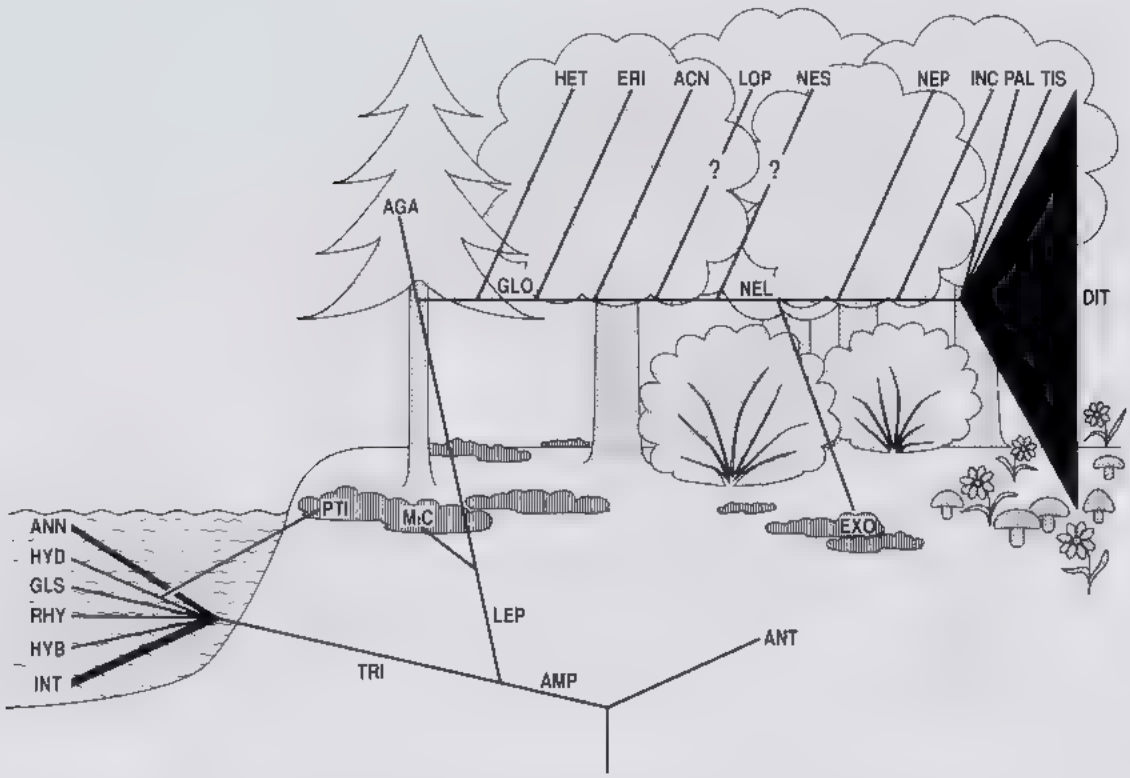


FIG 1 — Cladogram of basal amphiesmenopteran lineages superimposed on major habitat types, secondary habitat shifts may occur *within* terminal taxa ACN, Acanthopteroctetidae, AGA, Agathiphagidae, AMP, Amphiesmenoptera, ANN, Annulipalpia, ANT, Antliophora (= Mecoptera + Siphonaptera + Diptera), DIT, Ditrysia, ERI, Eriocraniidae, EXO, Exoporia, GLO, Glossata, GLS, Glossosomatidae, HYB, Hydrobiosidae, HYD, Hydroptilidae-Hydroptilinae, HTB, Heterobathmiidae, INC, Incurvarioidea, INT, Integripalpia, LEP, Lepidoptera, LOP, Lophocoronidae, NEL, Neolepidoptera, NES, Neopseustidae, NEP, Nepticuloidea, PAL Palaephatidae, PTI, Hydroptilidae-Ptilocolepinae, RHY, Rhyacophilidae, TRI, Trichoptera; TIS, Tischeriidae

NIELSEN (1989), NIELSEN & KRISTENSEN (1996) and POWELL *et al.* (in press). Problems of formal classification are discussed by KRISTENSEN (*in* CARTER & KRISTENSEN, in press), it is here advocated that recognition of sub- and infraorders in the Lepidoptera should be discontinued.

Four primary lepidopteran clades are currently recognized the families Micropterigidae, Agathiphagidae and Heterobathmiidae, and the high-rank taxon Glossata comprising all other Lepidoptera, *i.e.*, 99 9% of the described species. SHIELDS (1993) and IVANOV (1994) considered the first splitting event traceable in extant Lepidoptera to have been between the Agathiphagidae and all other clades, but for reasons discussed elsewhere (KRISTENSEN, 1984b, in press a, KRISTENSEN & SKALSKI, in press), I believe the interrelationships should be represented as Micropterigidae + (Agathiphagidae + (Heterobathmiidae + Glossata))(Fig 1), the latter phylogeny has also received support from analyses of 18S rDNA (WIEGMANN, 1994)

Source MNHN Paris

The Glossata comprise six basal clades the families Eriocraniidae, Acanthopteroctetidae, Lophocoronidae, Neopseustidae, and the high-rank taxa Exoporia (Mnesarchaeoidea + Hepialoidea) and Heteroneura (all remaining Glossata) A recent analysis of the interrelationships of these clades, based on 47 characters in skeletal and "soft" anatomy, has yielded a single most parsimonious solution (Figs 1-2), *viz* , Eriocraniidae + (Acanthopteroctetidae + (Lophocoronidae + (Neopseustidae + (Exoporia + Heteroneura)))) A suprafamilial taxon "Dacnonypha" comprising the Eriocraniidae, Acanthopteroctetidae and Lophocoronidae is therefore non-monophyletic and must be discarded A detailed presentation of the analysis, including evidence for the monophyly of each of the six clades, is given by NIELSEN & KRISTENSEN (1996) Names have been given to a selection of the high-rank clades that are recognized: the Exoporia + Heteroneura have long been known as the Neolepidoptera, the Neopseustidae + Neolepidoptera are called Myoglossata, and the name Coelolepida has now been applied (NIELSEN & KRISTENSEN, 1996) to the entity comprising all non-eriocraniid Glossata

As is well known, the vast majority (>98%) of the extant Lepidoptera pertain to the heteroneuran clade Ditrysia, four other heteroneuran basal clades are recognized, *viz.*, the Nepticuloidea (Nepticulidae + Opostegidae), Incurvarioidea (six families), Palaephatoidea (Palaephatidae only) and Tischerioidea (Tischeriidae only) The interrelationships within the Heteroneura remain unsettled, but the phylogeny represented as Nepticuloidea + (Incurvarioidea + (Palaephatoidea + Tischerioidea + Ditrysia)) may be best supported at present (KRISTENSEN & SKALSKI, in press)

The oldest known fossil moth is *Archeolepis manae* Whalley, 1985 from the Lower Jurassic, its systematic position within the Lepidoptera is unclarified The fossil record has so far contributed little to the dating of early splitting events within the "order" (*cf.* below), but apparently reliably identified leaf mines of Ditrysia-Gracillariidae (LABANDEIRA *et al* , 1994) from the Middle Cretaceous (97 myr B P) are evidence that all major homoneurous and monotrysian lineages existed by that time

Life history patterns of non-ditrysian moths

It is unknown whether agathiphagid moths feed at all, but because of the absence of incisivus teeth on their mandibles it is unlikely that they utilize solid foods In contrast, the adult insects belonging to the two other pre-glossatan families feed on pollen or (in the case of some micropterigids from New Caledonia and [D LEES, personal communication] Madagascar) fern spores The stem lineage (*sensu* Ax, 1987) of glossatan moths was characterized by the loss of mandibular function in the post-pharate adult, and the development of the coilable proboscis from the maxillary galeae Hereby adults of all higher Lepidoptera were rendered dependent on fluid nutrients exclusively

Larval biologies in the non-glossatan grade are diverse As noted above, micropterigid larvae are soil dwelling, they feed on foliose liverworts, plant debris and/or fungus hyphae, and they are restricted to quite moist habitats Agathiphagid larvae are miners in kauri pine (Araucariaceae) seeds, oviposition is believed to take place while the seed is still in the cone, but the larval development and pupation is completed in the seed after it has fallen to the ground Larval heterobathmiids are leaf miners in *Nothofagus* (Fagaceae), apparently restricted to the deciduous taxa, the fully grown larva vacates the mine, falls to the ground and pupates in a cocoon in the earth

Source MNHN Paris

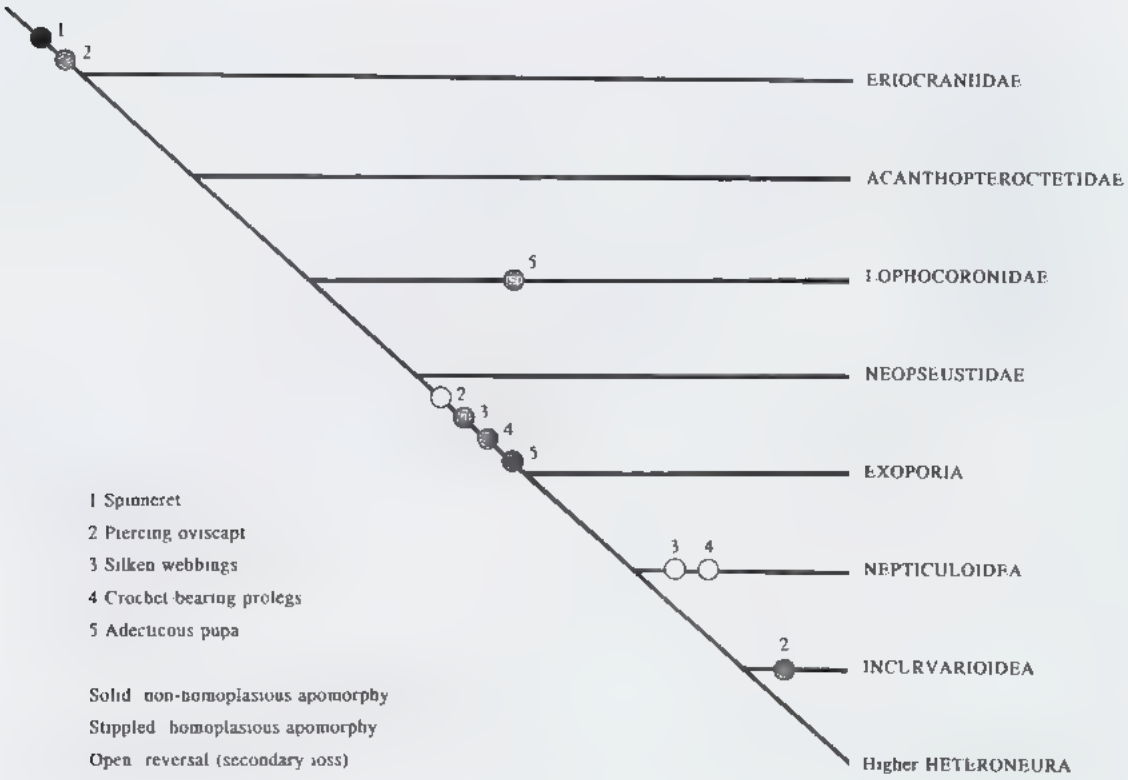
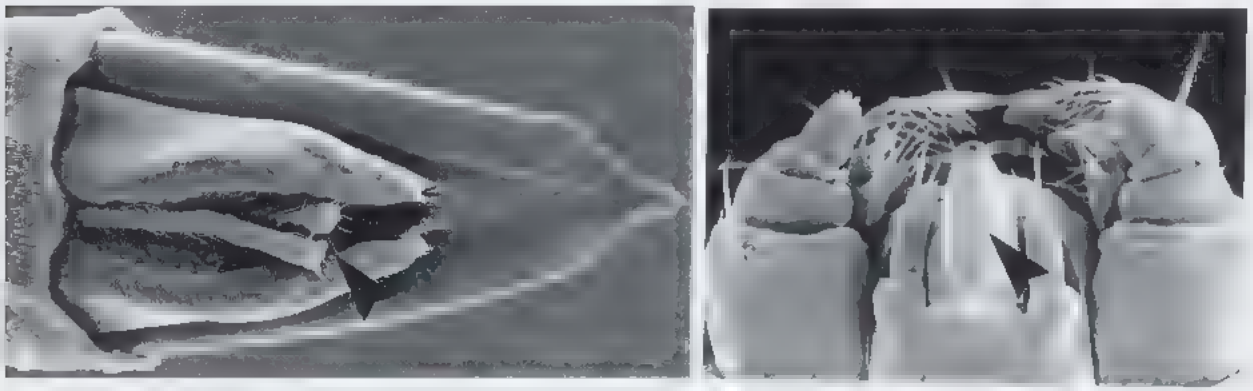


FIG 2 Cladogram of basal lineages within Lepidoptera Glossata, with some key evolutionary events indicated

Among the basal Glossata the Eriocraniidae have a larval biology which is remarkably similar to that of the Heterobathmiidae in the pre-glossatan grade, they are leaf miners, almost exclusively restricted to trees in the Fagales (with a few occurrences on the Rosales), and they pupate in a cocoon in the earth However, whereas in the Heterobathmiidae the egg is deposited on the host leaf surface (and covered by a secretion) eriocraniid eggs are inserted in pockets in the leaf, cut by the female's piercing oviscapt (Fig 3) The sole acanthopteroctetid for which the life-history is known is a leaf miner in *Ceanothus* (Rhamnaceae), and it pupates in a cocoon in debris under the host (DAVIS & FRACK, 1987) Immature Lophocoronidae and Neopseustidae are unknown, but since females of the former have a piercing "*Eriocrania*-type oviscapt" (as have the Acanthopteroctetidae), it is believed that their larvae are similarly endophagous, probably leafminers Neopseustid females also have what appears to be a kind of piercing

Source MNHN, Paris

postabdomen (clearly distinct from the "*Eriocrania*-type", though perhaps derivable from it), hence immatures in this family may also be endophagous



FIGS 3-4 — Eriocraniid structures (SEM), illustrating putative groundplan features of Lepidoptera-Glossata **3**: *Dyseriocrania subpurpurella* (Haworth, 1828) female abdominal apex (ventral view) "*Eriocrania*-type" piercing oviscapt with lateral "saws", arrow indicates cloacal opening **4**: Same larval mouthparts (ventral view), showing articulated spinneret (arrow)

It is in the Exoporia that one first encounters the "typical lepidopteran caterpillar" with its complement of five pairs of musculated, crochet-bearing prolegs (Figs 6, 7), borne on abdominal segments III-VI and X Mnesarchaeoid larvae are "soil animals" which live in silken tunnels among bryophytes etc, often together with micropterigid larvae, and they are "completely unspecialized phytophages" (GIBBS, 1979) The biology of the "smaller hepialoid families" is poorly known, but larvae of *Ogygioses* (Palaeosetidae) occur in habitats similar to those of mnesarchaeids (DAVIS *et al*, 1995, HEPPNER *et al.*, 1995) Hepialid larvae (see GREHAN, 1989) have diverse habits Many make tunnels in the soil or construct silken galleries among litter, feeding on roots or leaves of a variety of plants including pteridophytes, gymnosperms and angiosperms, some are root/stem/branch borers Fungivory is probably widespread, and a transition from fungivory to phytophagy during larval ontogeny has been recorded in a number of cases

Most of the known representatives of the smaller heteroneuran lineages feed on living angiosperms, and so did in all probability their last common ancestor Nepticuloid females deposit their eggs on the surface of the host plant, but all larvae are endophagous nepticulids mostly leaf-miners, but a few are stem-miners, the few opostegids with known biologies are leaf/petiole- or branch/stem/trunk (cambium)-miners (DAVIS, 1989) The hostplant spectrum of this superfamily, and that of the Incurvaroidea, comprise a large array of (mostly dicot) families Incurvarioid females have piercing oviscapts somewhat reminiscent of the "*Eriocrania*-type" In the apparently ancestral life-history in the superfamily early larval instars are leaf-mining, while the older instars live on the ground, bearing a portable case constructed from the excised walls of the mine and feeding on living or dead plant material In some Adelidae even the first-instar larvae are free-living soil-animals, but it is remarkable that these moths also have retained the endophytic oviposition mode The permanently endophagous (flower/fruit/stem-boring, gall-

Source MNHN, Paris

making etc) larvae of the cecidosid/prodoxid clade surely exemplify a pattern which is derived within the superfamily The few known larvae of Palaephatoidea are initially leaf-miners, later living between two leaves (joined by silk along the margins) and feeding on their the inner epidermis and parenchyme cells (E S NIELSEN, pers com), the Tischerioidea are consistently leaf miners In neither of these taxa are the females equipped for endophytic oviposition

The major question: derivation of angiosperm feeding in basal moth lineages

Accepting the above conclusion that the soil-dwelling, detritophagous larvae of extant Micropterigidae are overall similar to those of ancestral Amphiesmenoptera, it is pertinent to ask how long this larval life style persisted in the sister lineage of the micropterigids Do such exoporians as mnesarchaeids or palaeosetids spend their larval stage in the same environment as micropterigids because all their lepidopteran ancestors did so? In this case all other homoneurous moth lineages (with known life histories) would have made independent transitions from the soil to arboreal habitats. Or was this transition made in the stem-lineage of Agathiphagidae + (Heterobathmiidae + Glossata), with the soil-dwelling exoporians representing an evolutionary reversal? By the way, Agathiphagid larvae are classified as "arboreal" in acceptance of the aforementioned inference that the eggs are laid while the seed is still in the cone (and perhaps the initial part of the larval life is spent here before the seed falls to the ground) Also, the Exoporia are here regarded as primarily soil-dwelling, and habitat shifts *within* this clade are disregarded in the present context

With the availability now of a largely resolved phylogeny for the basal moth clades, parsimony speaks clearly in favor of the latter solution (Fig 1) While it requires only two steps, the former requires at least five transitions from the soil to arboreal habitats, *viz.*, in the stem lineages of Agathiphagidae, Heterobathmiidae, Eriocraniidae, Acanthopteroctetidae and Heteroneura It almost certainly requires at least a sixth transition also, since although immature Lophocoronidae are unknown, the presence in lophocoronid females of a piercing "*Eriocrania*-type" oviscapt is strong evidence that these insects have endophytic larvae Even the female postabdomen of the Neopseustidae has a structure which presumably reflects a boring/rasping oviposition mode that would be unexpected if the larvae lived in soil/periphyton interstices

Judging from the cladogram of extant moths, angiosperm-feeding was first adopted in the stem-lineage of the Heterobathmiidae + Glossata And if the Agathiphagidae are indeed the sister-group of all other non-micropterigid Lepidoptera, then parsimony would favour the notion that the last common ancestor of these two lineages had canopy-living and -ovipositing moths The inference is straightforward that the shift has come about through the utilization of arboreal pollen sources by adults of early moths with soil-dwelling larvae, the same kind of ontogenetic habitat shifts are illustrated by some extant micropterigids

How does the fossil record comply with the notion of angiosperm feeding in extant pre-glossatan moths (*viz* , heterobathmiids) being primary? A reliable fossil record of eudicot angiosperms (which include the hosts of extant Heterobathmiidae and Eriocraniidae) dates back no futher than the Early Cretaceous (CRANE *et al* , 1995) ROZEFELDS (1988) interpreted gallery mines in Jurassic pteridophyte leaves from Australia as being due to Heteroneura-Nepticulidae, but the evidence is inadequate (no frass was detectable in the mines) and the identification was discarded by KRISTENSEN & SKALSKI (in press) However, the assignment of one pre-Cretaceous

Source MNHN Paris

moth, *viz.*, the Upper Jurassic *Protolepis cuprealata* KOZLOV, 1989 (Fig 5) to the Glossata is still being upheld by KOZLOV (see KRISTENSEN & SKALSKI, in press, POWELL *et al.*, in press)

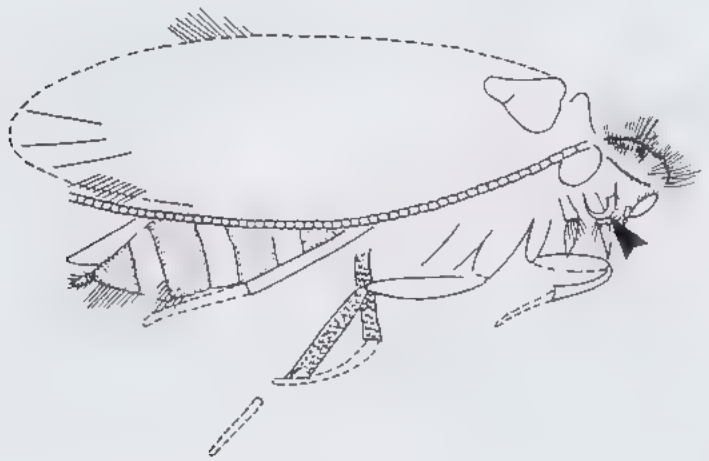


FIG 5 — *Protolepis cuprealata* Kozlov, 1989, drawing accompanying original description, the arrow indicates the problematical mouth appendage: proboscis or maxillary palp?

Two reservations are in order Firstly there is, of course, a theoretical possibility that the fossil history of eudicots may be considerably older than documented by fossils, though the counter-evidence presented by CRANE *et al* is seemingly strong (absence of characteristic eudicot pollen in numerous rich pre-Cretaceous palynofloras from both hemispheres) Secondly, I remain unconvinced about the glossatan nature of *Protolepis* In particular I consider it likely that the curved mouth appendages are the maxillary palps rather than haustellate galeae, conditions in extant primitive glossatans would lead one to expect the former to be much more prominent formations than the latter It therefore remains a real possibility that the larva of the last common ancestor of Heterobathmiidae and the Glossata was a leaf miner in a fagacean host, though the possibility of later host switches in the Heterobathmiidae and/or the Eriocraniidae cannot be ruled out In any case structural modifications linked to the leaf-mining habit has progressed to different stages in these early angiosperm-feeders, for example, eriocraniid larvae have lost the thoracic legs, while these are still retained in heterobathmiids and acanthopteroctetids

Larval spinning and larval prolegs

Silken threads play a major role in the behavioral diversification of larvae in both amphiesmenopteran "orders" In the first differentiated, "pre-exoporian", lineages of the Lepidoptera, however, the only use made of the larval silk is for the spinning of the cocoon

Source MNHN Paris

FIGS 6-7 — *Mnesarchaea* sp (Exoporia-Mnesarchaeidae), crochet-bearing larval prolegs (SEM) illustrating probable neolepidopteran groundplan configuration **6**: Side view **7**: ventral view

before pupation, such silken cocoons occur in all the known families of this grade (remember that lophocoronid and neopseustid larvae are unknown), Agathiphagidae excepted Indeed, Agathiphagid pupae remain in the mined seed and have no need for other coverings However, the labial glands of the larvae are exceedingly large and their secretion presumably plays a major role in the formation of the hard inner lining of the pupal cell It is perhaps surprising that the development of the "spinneret" (the slender, passively movable process with the spinning gland aperture on the apex, Fig 4), which is such a prominent groundplan autapomorphy of the Glossata, was not - as far as presently known - initially associated with any marked change in spinning behavior

The use of silk for construction of galleries or other kinds of webbings in which larvae live is first encountered in the Neolepidoptera Remarkably, as noted above, it is in the same clade that one first encounters the crochet-bearing proleg (Figs 6, 7) of the "typical caterpillar" It is commonplace to think of these prolegs as an adaptation to clinging to plant surfaces (e g STRONG *et al* , 1984) It must be emphasized, however, that the substrates on which exophagous exoporian larvae move are silk webbings, rather than plant surfaces Larvae of ancestral Exoporia probably lived in spinnings among periphyton as described above for mnesarchaeids, and as noted by GREHAN (1989) leaf-feeding hepialid caterpillars usually forage in the immediate vicinity of their tunnel/gallery entrances Prolegs and crochets are absent (secondarily lost,

Source MNHN Paris

according to the most widespread view which I endorse) in the endophagous larvae of the Heteroneura-Nepticuloidea Prolegs are also poorly developed in the Incurvarioidea and Tischerioidea, but more or less distinct crochets are generally retained in both superfamilies, in case-bearing late-instar incurvarioid larvae the crochets engage in a silken lining of the case (SCHELLAUF, 1994), and tischerioids line their blotch mines with silk

Since silken webs and crochet-bearing prolegs evolved on the same internode on the lepidopteran cladogram (as constructible on the basis of known, extant taxa), this cladogram cannot in itself provide unambiguous evidence that the prolegs evolved as an adaptation to moving on a silken web, however, functional anatomy corroborates this assumption (SCHELLAUF, 1994). The fact that the convex curvature of a caterpillar crochet faces the substrate, and the tip therefore is directed away from the latter in the resting position (*i.e.*, with the plantar muscle uncontracted) is most readily explicable, if the crochet evolved in response to a selective pressure for enhancing grips on a silken webbing If the initial selective regime had been for enhancing movement on a smooth plant surface, one would rather have expected crochet curvature to have been reversed. The crochet-bearing prolegs are, therefore, apparently an exaptation to clinging onto plant surfaces, which surely is their principal function in the bulk of the Lepidoptera In many cases the proleg grip is preceded by the larva fastening silken threads on the substrate, and/or the distal proleg configuration is profoundly modified, as in the "Macrolepidoptera-type" proleg

Eclosion mode

The stem lineage of the Neolepidoptera is characterized by another notable behavioral innovation the exarate, decticous pupal type is replaced by the adecticous obtect type Functionally the loss of mobility of the pupal appendages is compensated for by the development, in the neolepidopteran ground plan, of a spinose pupal abdomen which permits the pharate adult to wriggle out of the pupal enclosure prior to pupation

Though lophocoronid and neopseustid pupae are unknown, examination of the adult structure permits important inferences Adult neopseustids have well-developed mandibular muscles, and it has therefore been concluded that their pupae are decticous, moreover, it is inferred that they also are exarate, because all known decticous pupal types are so Adult lophocoronids, on the other hand, have the mandibular musculature completely reduced, and their pupae are thus necessarily adecticous However, no inference can be made as to whether they are obtect as in the Neolepidoptera, since adecticous exarate pupae are known elsewhere among endopterygotes.

The preferred phylogeny of the Glossata (NIELSEN & KRISTENSEN, 1996) necessitates the postulate that the adecticous pupae in Lophocoronidae and Neolepidoptera are independently evolved In the analysis the "cost" of making the origin of the adecticous pupa a unique event through switching the Lophocoronidae to the position as sister-group of the Neolepidoptera is three extra steps Evidently a future discovery of lophocoronid immatures will be significant in this context If lophocoronid pupae prove to be obtect and spinose like those of neolepidopterans, the support for the second phylogeny would be at least somewhat strengthened

It should be emphasized, however, that transitions from the decticous to the adecticous pupal type have occurred repeatedly among endopterygote insects, and homoplasy of this trait

Source MNHN Paris

within the Lepidoptera is not unexpected Functionally the transition appears easily explicable it frees the insect from retaining, up to the adult stage, the investment of precious proteins in the bulky mandibular musculature, which is used only during a very brief phase of the adult insect's life Thus, the breakdown of the mandibular musculature which has been observed in post-pharate eriocraniids (as in caddisflies), can begin during pupal life, whereby an earlier re-use of components for *e.g* oocyte growth or genital-duct secretions is made possible

THE TRICHOPTERAN LINEAGE

The shift of larval habitat from the soil into the genuinely aquatic environment was arguably the key innovation in the early evolution of the caddisfly lineage The initial step has probably not been a major one in an ecological sense, inasmuch as some "soil" habitats may well be characterized as at least semi-aquatic However, as far as known, all extant trichopteran larvae share one significant apomorphy which one would immediately interpret as an adaptation to the aquatic life-style the apneustic tracheal system Other trichopteran larval groundplan autapomorphies are regressive traits with less obvious functional significance greatly shortened antennae without any extrinsic musculature, single maxillary endite lobe and very delicate tentorium (the two lastmentioned states are parallelled in all non-micropterigid Lepidoptera)

More or less pronouncedly terrestrial larvae occur in a number of caddisfly lineages (the Palaearctic limnephilid genus *Enoicyla* is perhaps the best known example), but it has not been questioned that these larvae are all secondarily non-aquatic Importantly, as noted by HINTON (1958), the *Enoicyla* larva is indeed apneustic

WIGGINS (1984) drew attention to the life-style of micro-caddisflies of the *Ptilocolepus* group as being particularly close to that which might be inferred for the amphiesmenopteran ancestor of the trichopteran lineage Their larvae are associated with wet liverworts, as are most micropterigid moths in the *Sabatinca*-group of genera, and they "crawl over dripping tiers of these plants, as often out of water as in it" This caddisfly taxon (comprising the W Palaearctic *Ptilocolepus* and the Amphipacific-Holarctic *Palaeagapetus*) is currently ranked as a subfamily in the Hydroptilidae (MARSHALL, 1979) It remains debatable, however, whether the diagnostic traits it shares with the Hydroptilinae are actually apomorphies They are (1) free-living 1st-4th larval instars, with tergal sclerites on thorax and I-VIII, (2) "hypermetamorphic" last (5th) instar larva with swollen abdominal segments and tergal sclerites on all thoracic segments, living in a two-valve case made from leaf fragments In the light of the uncertainty about the affinities of the *Ptilocolepus*-group it does seem pertinent to ask again whether they could indeed be the sister-group of all other caddisflies, and their larvae therefore primarily semiaquatic However, even these larvae are apneustic (I have examined serial sections of the thorax and first abdominal segments of larval *Palaeagapetus* without finding any trace of functional spiracles), and thereby conform with the inferred trichopteran groundplan On this basis the conclusion seems inescapable that ptilocolepine caddises are derived from ancestors with fully aquatic immatures, as hitherto presumed

How does one recognize an adult caddisfly?

The trichopteran groundplan autapomorphies mentioned above are all in the larval stage Indeed, and in striking contrast to the Lepidoptera, adult Trichoptera are actually quite difficult

Source MNHN, Paris

to diagnose as such This impression is amply supported by an examination of SCHMID's (1989) somewhat detailed reconstruction of the integumental structure of the ancestral caddisfly

Alleged autapomorphies hitherto identified in the groundplan of adult Trichoptera (KRISTENSEN, 1991/1994) include (1) prelabio-hypopharyngeal lobe forming large "haustellum" (protrusible/eversible by blood pressure) with intricate system of canals (formed by modified microtrichia) enabling uptake of fluid nutrients, (2) clypeolabral articulation and extrinsic labral muscles absent (parallelism with Mecoptera), (3) true mandibular articulation absent (parallelism with Lepidoptera-Glossata) Not all of these can be upheld, however The haustellum itself remains a good autapomorphy of adult caddis flies, indeed it is the only at all "strong" one, but the complement of surface canals cannot These canals were identified in the Integripalpia by CRICHTON (1957), but while this author found all examined non-Integripalpia (Annulipalpia-Polycentropodidae excepted, most likely due to secondary modification) to have a "granulose" surface texture devoid of canals, KLEMM (1966) construed the microtrichia on the *Rhyacophila* haustellum to be aligned in a manner to form canals somewhat comparable to those of the Integripalpia It is on this basis that I had attributed a canal system to the trichopteran ground plan However, by subsequent SEM investigation I have discovered that at least the Hydroptilidae (Ptilocolepinae included) and Glossosomatidae have no kind of longitudinal/radial alignment of haustellum microtrichia, hence no canal system Instead, in these taxa the haustellar microtrichia (which are simple) are arranged along transverse crests (Figs 8-9) Since this arrangement is reminiscent of the spine armature in the infrabuccal pouches on the hypopharyngeal surface in the non-glossatan moths, it most probably represents the ground plan state in the Amphiesmenoptera and hence in the Trichoptera.

It is similarly necessary to discard the obliteration of the "clypeolabral articulation" as a trichopteran autapomorphy I have now found that a well-developed clypeolabral membrane is actually retained in several members of the Annulipalpia But all the caddisflies I have sectioned (including representatives of all primary clades, cp below) are devoid of extrinsic labral muscles, hence the loss of these muscles may well have been a unique event in the trichopteran stem lineage, *i.e.*, it can upheld as an autapomorphy of the group So can the loss of genuine cranio-mandibular articulations, associated with the absence of mandibular function in post-pharate adult caddisflies

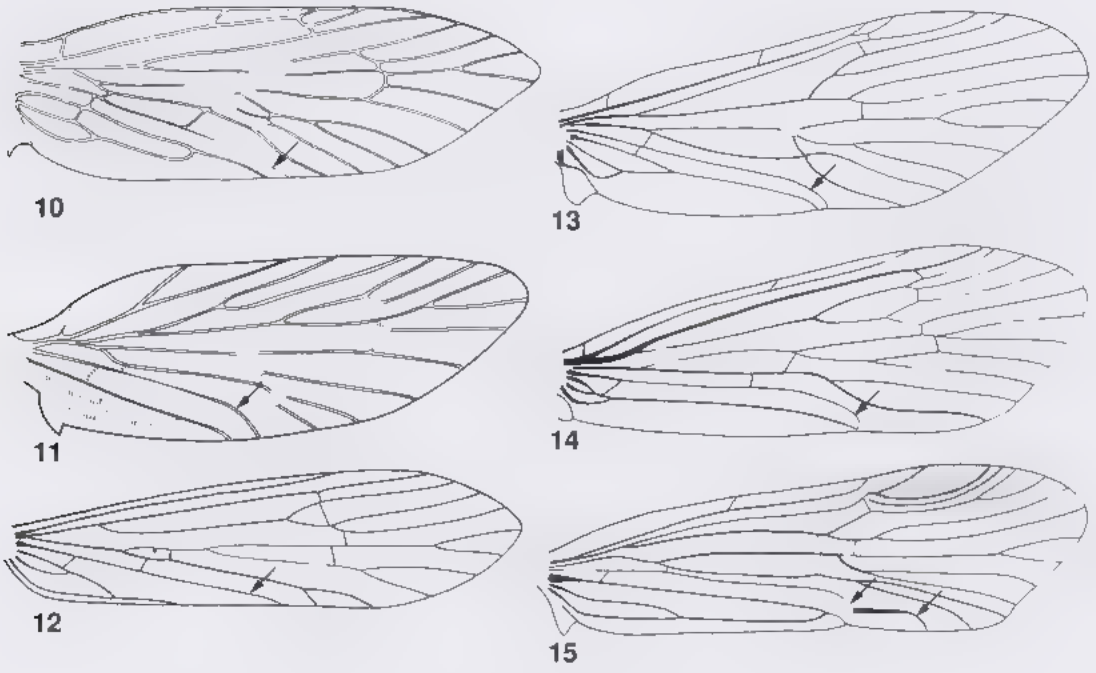
It is important to emphasize again that none of the autapomorphies hitherto identified in adult caddisflies are easily observable, and in particular that they are unlikely to be of use in the case of fossils However, attention shall here be drawn to a venational character which deserves scrutiny as a potential aid in diagnosing Trichoptera the distal course of the forewing CuP In most Lepidoptera, including all homoneurous lineages, this vein (often quite weak) is almost straight or at most smoothly curved (Figs 10-11) On the other hand, in all extant basal clades within the Trichoptera it is commonplace that the apical part of this vein is abruptly bent towards the wing margin (Figs 13-15), it therefore reaches the margin only a short distance beyond the anal vein (*i.e.*, the apical "stem" of the "double-Y" formed by the fusion of 1A + 2A + 3A), and it frequently even fuses with the latter (Fig 14) A modification of this pattern is characteristic of the Hydrobiosidae (SCHMID, 1989) In this family (Fig 15) the portion of CuP beyond the bend again becomes more or less parallel with CuA2, conferring upon CuP an "undulated" configuration, in some cases (*Australochorema* Schmid and *Apsilochorema* Ulmer are pronounced examples) the apicalmost part of CuP is again sharply bent, so a "double

Source MNHN, Paris

FIGS 8-9 – *Ptilocolepus granulatus* (Pictet, 1834), (Trichoptera Hydroptilidae-Ptilocolepinae), adult mouthparts (SEM, frontal view) **8.** Haustellum with aligned microtrichia, haustellar base largely covered by maxillary lobes (interpreted as galeae), apex of labrum visible on top **9:** Microtrichia lines at higher magnification

undulation" of the vein arises Most extant and extinct Mecoptera have a straight/smoothly curved CuP, and so do many fossils classified as stem-lineage panorpoids (see WILLMANN, 1989), hence this state is presumably the plesiomorphic one But the character is obviously homoplasious (which is unsurprising, given its simplicity) For example, some extant Mecoptera-Bittacidae do have a marked bend, and so do the Permian Amphiesmenoptera-Microptysmatidae, which have a six-branched Rs and therefore presumably can at most belong to the amphiesmenopteran stem-lineage By the way, WILLMANN (1989) would not exclude that the six-branched Rs could be a microptysmatid autapomorphy It is also easy enough to find examples of extant caddisflies in which the apical curvature of CuP is little pronounced or even non-existent (Fig 12), presumably character reversals The CuP configuration can thus only be taken as an indication of whether a given amphiesmenopteran (extinct or extant) belongs to the trichopteran lineage, not an absolute proof

Source MNHN Paris



FIGS 10-15 Forewings of various Amphiesmenoptera (not drawn to scale), illustrating diversity in apical CuP-configuration (arrows) **10:** *Agathiphaga vitiensis* Dumbleton, 1952 (Lepidoptera-Agathiphagidae), note the pattern of vein branching/anastomosing in the pre-cubital wing area is surprisingly variable from one individual to the next in this taxon **11:** *Sabatinca calliarcha* Meyrick, 1912 (Lepidoptera-Micropterigidae), exemplifying a primitive moth with unusually strongly bent CuP **12:** *Psychomyia nomada* Ross, 1938 (Trichoptera Psychomyidae), a caddisfly with (?secondarily) straight CuP **13-15:** Caddisflies with CuP strongly bent apically, an auxillary ordina, groundplan autapomorphy **13:** *Rhyacophila torrentium* Pictet, 1834 (Rhyacophilidae) **14:** *Stenopsychodes tillyardi* Banks, 1939 (Stenopsychidae), showing subapical fusion of bent CuP and fused A veins **15:** *Australochorema rectispinum* Schmid, 1955 (Hydrobiosidae), showing "double undulation" of CuP [10-11 original, 12-15 from SCHMID (12 *Memoires de la Societe Entomologique du Canada* **125** 1983, 13 *Mémoires de la Societe Entomologique du Canada* **66**, 1970 14 The Canadian Entomologist **101** 187-224 1969, 15 *Bulletin de l'Institut Royale des Sciences Naturelles de Belgique*, **59**, Supplement, 1989)]

While NOVOKSHONOV & SUKATCHEVA (1993) opened a recent review by saying that "Caddisflies are common as fossils from the Permian onwards", the earliest concrete evidence for the existence of the trichopteran lineage is actually from the Lower Jurassic, and it is indirect the existence of the sister-lineage (namely the lepidopteran *Archaeolepis*) I do consider it very likely that the split between the lepidopteran and trichopteran lineages took place at least in the Triassic, but reliable evidence will be difficult to obtain The assignments of the Protomeropidae, Microptysmatidae, Cladochoristidae, Liassophilidae, Prorhyacophilidae, and Dysoneuridae to the Trichoptera (CARPENTER, 1992, NOVOKSHONOV & SUKATCHEVA, 1993) have all been unfounded within the framework of phylogenetic systematics Whereas the many costo-subcostal crossveins and/or high number (>4) of Rs branches in the four firstmentioned taxa would seem to

Source MNHN, Paris

preclude that these can even belong in the amphiesmenopteran "crown-group" (Amphiesmenoptera *sensu* HENNIG), the two lastmentioned may indeed comprise members of the trichopteran lineage, they may, however, equally well include stem-lineage Amphiesmenoptera and stem-lineage Lepidoptera The same is true of the Necrotauliidae, some Jurassic members of which have the forewing CuP strongly bent apically, these taxa, therefore, can with some justification be talked of as caddisflies The Upper Jurassic/Lower Cretaceous *Necrotaulius tener* Sukatcheva, 1990 reportedly has a short, annulated apical segment of the maxillary palp, hence it has been assigned to the stem-lineage of the Annulipalpia *s. str.*, and if this palp character is correctly interpreted this assignment is justifiable It would not be surprising if the extant basal caddisfly clades were indeed differentiated by the Jurassic/Cretaceous boundary

Basal trichopteran clades and the problems of their interrelationships

Six basal clades are currently recognized within the Trichoptera The largest are the Integripalpia *s.str.* whose larvae are tube-case makers, and the somewhat less species-rich Annulipalpia *s.str.* (= Curvipalpia) whose larvae are net-spinners/retreat makers The remaining caddisflies are overall generalized taxa, now grouped into four families the Rhyacophilidae and Hydrobiosidae (larvae free-living, carnivorous), the Hydroptilidae (larvae free-living, except last instar which make "purse-cases") and the Glossosomatidae (larvae "saddle-case" makers) These four families are believed by some to constitute a monophylum "Spicipalpia" which is the sister group of the Annulipalpia (WEAVER, 1984, WEAVER & MORSE, 1986), alternatively the "spicipalpian" families have been seen as an assemblage which is paraphyletic in terms of the Annulipalpia (SCHMID, 1989), the Integripalpia (ROSS, 1967), or both (cladograms obtained by FRANIA & WIGGINS, WIGGINS, pers com) In spite of major recent efforts (FRANIA & WIGGINS, in press) no single convincing phylogeny of the caddisfly clades has so far been obtained

Considerations of ancestral larval habits/habitats and pupation modes have played a major role in the current debate over these unresolved interrelationships (for an entry into the pertinent literature see WEAVER, 1992a, 1992b, WIGGINS, 1992) In one scenario (WIGGINS & WICHARD, 1989) ancestral caddisfly larvae are believed to have been free-living (at least in earlier instars) and inhabiting cool lotic waters, in another (WEAVER & MORSE, 1986) they are believed to have lived in silken tubes in humus/detrital mats in the lentic or "lotic-depositional" zone

Both scenarios may well contain elements of the truth Given the assumption that ancestral Amphiesmenoptera (and indeed panorpoid endopterygots) were soil-dwelling, it is difficult to see how the transition into lotic waters could have taken place via any other habitat than that envisaged in the WEAVER/MORSE theory, though the last common ancestor of the trichopteran crown group apparently, as noted above, has had a more fully aquatic larva than those of extant amphibious spicipalpians like the Hydoptilidae-Ptilocolepinae On the other hand it must be strongly emphasized, that contrary to WEAVER's claims (1992b), evidence from Lepidoptera lends no support to the notion that trichopteran larvae in silken tubes represent an ancestral amphiesmenopteran life-style WEAVER referred to the tube-dwelling Exoporia, but since this lineage did not arise until the *eighth* splitting event traceable among extant Lepidoptera, they have little relevance for the question of ground plan conditions in the group, let alone the Amphiesmenoptera As noted earlier, none of the more basal lepidopteran lineages have larvae that are known to live in silken galleries out-group evidence therefore supports that the free-living caddisfly larvae represent the ancestral life-style

Source MNHN Paris

Given the long-standing interest in the behavioural diversity of caddisfly larvae, it is much to be hoped that forthcoming renewed efforts will soon result in a trustworthy trichopteran phylogeny upon which this diversity can be mapped and interpreted

ACKNOWLEDGEMENTS

This paper is dedicated to Professor T YASUDA, University of Osaka, on the occasion of his retirement A largely similar version of this article appears in Japanese in T YASUDA (ed) *Biology of Microlepidoptera* I am grateful to Professor G B WIGGINS and Dr H MALICKY for useful information and for material of Hydroptilidae-Ptilocolepinae Dr E S NIELSEN provided useful information about palaephatid immatures Skilled assistance with the illustrations was provided by Mr G BROVAD (printing) and Ms B Rubaek (line drawings).

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Phylogeny and Evolution of the Larval Diet in the Sciaroidea (Diptera, Bibionomorpha) since the Mesozoic

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ABSTRACT

The larvae of the Keroplatidae exhibit diverse trophic specializations being either ferocious predators, killing their prey by way of toxic diffuse nets, or fungivorous insects, spinning sheet-like webs to gather the spores of polyporous fungi Previous studies of trophic specializations of larvae in Sciaroidea have been based up to now on inference from morphology or on empirical demonstration These have led to the general notion that fungivory is ancestral for the Sciaroidea and predation ancestral for the Keroplatidae New phylogenies for Sciaroidea and Keroplatidae are proposed here, the Cecidomyiidae seem to be the sister-group of all other families Seven attributes are mapped on the cladograms endobiosis/epibiosis, fungivory other diets, presence or absence of silk secretion predation/sporophagy, labial secretion pH < 3 net like/sheet-like web, optobiosis/cryptobiosis. It is concluded that fungivory and silk secretion are ancestral for Sciaroidea and predation ancestral for the Keroplatidae while sporophagy is a specialization derived from predation Epibiosis, with its cryptobiotic specialization, the net-like web and the highly acid pH, are also apomorphic for Keroplatidae Sporophagy, optobiosis, sheet-like web and less acid pH are correlated apomorphic traits for the tribe Keroplatini The fossil and biogeographical data allow dating most of these specializations back to at least the Lower Cretaceous

RESUMÉ

Phylogenie et evolution du regime alimentaire des larves de Sciaroidea (Diptera, Bibionomorpha)

Les larves de Keroplatidae presentent un regime alimentaire tres contraste, puisque les unes sont de redoutables predateurs qui tuent leurs proies au moyen d'une salive toxique repandue dans une toile de chasse, tandis que les autres sont infeodees aux Polypores, dont ils recueillent les spores dans une toile de récolte La question des specialisations trophiques chez les larves de Sciaroidea a jusqu'ici été abordée par des voies empiriques ou morpho-anatomiques, qui on conduit a penser notamment que la mycophagie etait ancestrale pour les Sciaroidea et la predation ancestrale pour les Keroplatidae De nouvelles phylogenies des Sciaroidea puis des Keroplatidae, sont proposées dans ce travail , les Cecidomyiidae apparaissent comme le groupe-frere de l'ensemble des autres familles de Sciaroidea Sept attributs sont superposes aux cladogrammes endobiose/epibiose, mycetophagie *s l* /autres regimes secretion de soie ou non predation/sporophagie, secretion labiale a pH < 3, toile en reseau nappe, optobiose/cryptobiose On est amene à conclure que la mycetophagie et la secretion de soie sont ancestrales pour les Sciaroidea et la predation ancestrale pour les Keroplatidae, tandis que la sporophagie de ces derniers est une specialisation developpée à partir de la predation L'epibiose avec sa specialisation en cryptobiose, la toile en reseau et le pH hautement acide, sont egalement plesiomorphes pour les Keroplatidae Sporophagie, optobiose, toile en nappe et pH moins acide sont des apomorphies correlees de la tribu des Keroplatini La datation de l'ensemble des cladogrammes par les fossiles

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MATILE L , 1997 — Phylogeny and evolution of the larval diet in the Sciaroidea (Diptera Bibionomorpha) since the Mesozoic *In* GRANDCOLAS, P (ed), The Origin of Biodiversity in Insects Phylogenetic Tests of Evolutionary Scenarios *Mém Mus. natn. Hist nat.*, **173** 273-303 Paris ISBN . 2-85653-508-9

Source MNHN Paris

et les données biogéographiques permet en outre d'attribuer à la plupart de ces spécialisations un âge au moins Crétacé inférieur

INTRODUCTION

The larvae of the Keroplatidae, a family of Sciaroidea, have highly diverse food preferences. Some are formidable predators, killing their prey by means of a toxic highly acidic saliva dispersed on a trapping net-like web, while others are live under bracket-fungi (Polyporaceae), where they gather spores in a sheet-like, less acidic web. Some lead a cryptic life, deeply hidden at maximum darkness and humidity (a way of life for which the term *cryptobiosis* is proposed), while others, while they do not shun obscurity, are able to live more or less in the open (*optobiosis*) if necessary.

Trophic specialization of the larvae of Sciaroidea has up to now been addressed empirically (KRIVOSHEINA, 1969, LASTOVKA, 1972, JACKSON, 1974), or through morpho-anatomy (ZAITSEV, 1983, 1984a, b, MATILE, 1986). These works led to the conclusion that fungivory was ancestral for Sciaroidea, and predation ancestral for the Keroplatidae. LASTOVKA (1972), who noted that fungivory is most common, and therefore plesiomorphic, also assumed that predation probably evolved from sporophagy.

I propose in this paper to test these previous hypotheses in the light of phylogeny, to study attributes (in the sense of BROOKS & MCLENNAN, 1991) linked to food preference in the Sciaroidea, especially the Keroplatidae. The following questions will be addressed: What was the ancestral food of the Keroplatidae larvae? What was the ancestral condition of their web? Was their common ancestor a cryptobiont or an optobiont? Moreover, it has been demonstrated by historical biogeography and by fossil data, that Keroplatidae already existed at least in the Upper Jurassic (MATILE, 1990; GRIMALDI, 1990, EVENHUIS, 1994). I shall try to date the appearance of these attributes by means of fossil and palaeogeographic data.

According to some authors who have recently addressed the problem of the classification of the Sciaroidea (or Mycetophiloidea), the superfamily consists of three families only: Mycetophilidae, Sciaridae and Cecidomyiidae (WOOD & BORKENT, 1989, COLLESS & MCALPINE, 1991) - the last two being sister-group to the first (WOOD & BORKENT, 1989). In a recent paper (OOSTERBROEK & COURTNEY, 1995), the superfamily is suppressed as such and included in the Bibionoidea, while the Mycetophilidae, Sciaridae and Cecidomyiidae show the same relationships proposed in WOOD & BORKENT's paper. Nevertheless, in all three of these papers (Fig. 1), the paraphyly of the adopted concept of "Mycetophilidae" is explicitly recognized.

On the other hand, authors who have treated fossil as well as recent taxa recognize more readily a group of "Mycetophilidae" families, sister-group to the Sciaridae, and then sister-group to the Cecidomyiidae, this last family treated by some as the superfamily Cecidomyioidea (ROHDENDORF, 1964, 1974, KOVALEV, 1987a, SHCHERBAKOV *et al.*, 1995). Many authors have treated the subfamilies of EDWARDS (1925) - Ditomyiinae, Diadocidiinae, Keroplatinae, Bolitophilinae, Mycetophilinae, Lygistorrhininae and Sciarinae - as of family rank like the Cecidomyiidae, but phylogenetically oriented papers are scarce (HENNIG, 1954, 1968, 1973, MATILE, 1986, 1990, AMORIM, 1992, Fig. 1). I have pointed out elsewhere (MATILE, 1993) that it might not be phylogenetically sound to use only three families "in keeping with North American tradition" (WOOD & BORKENT, 1989), neither do I feel dogmatic in thinking "that a

Source: MNHN, Paris

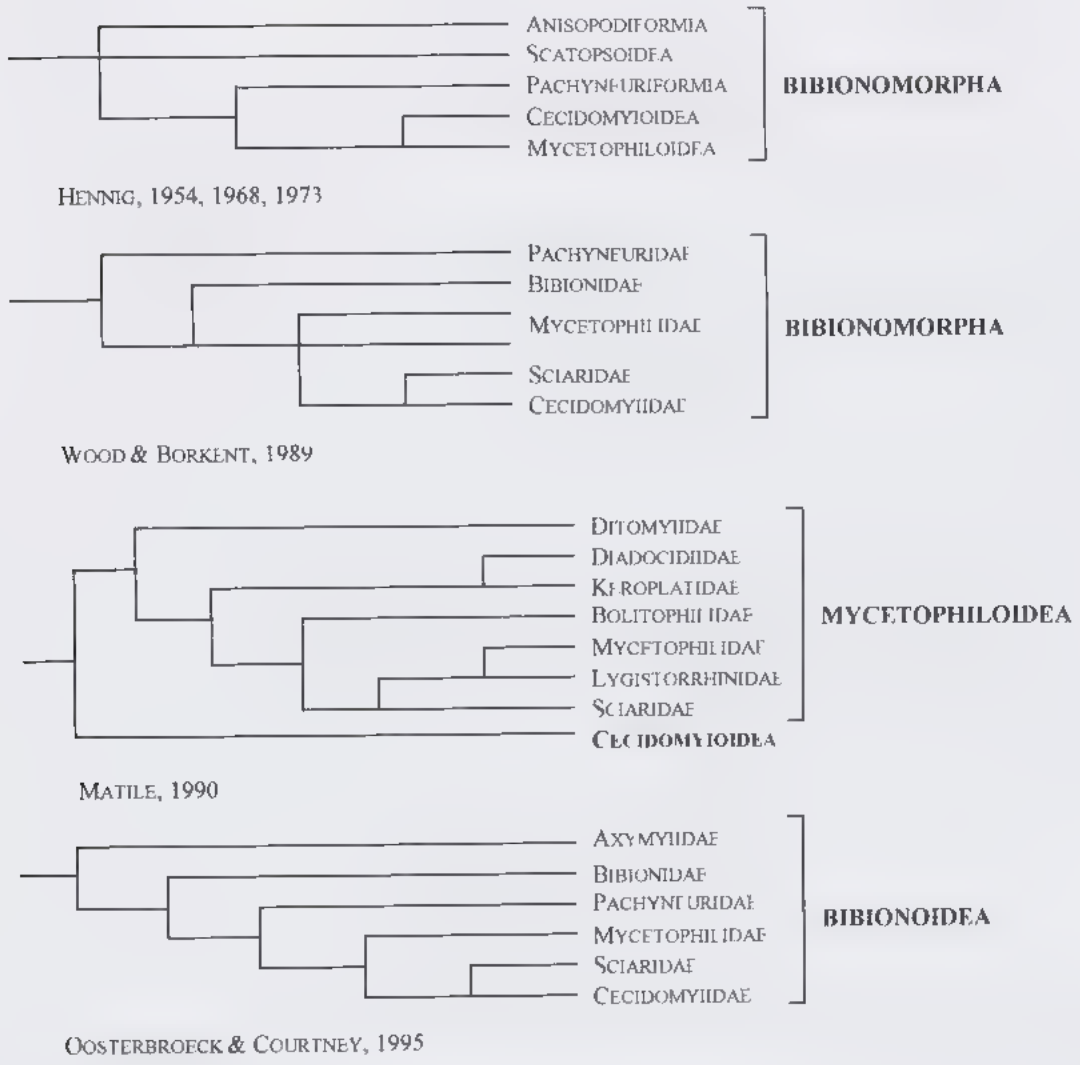

FIG 1 — Hypotheses on the phylogeny of the Bibionomorpha, or the Mycetophiloidea + Cecidomyioidea according to HENNIG (synthesis of 1954, 1968, 1973) WOOD & BORKENT, 1989 MATILE, 1990 OOSTERBROECK & COURTNEY, 1995

paraphyletic taxon cannot be a perfectly good one" (COLLESS & MCALPINE, 1991), especially for the kind of analyses presented here or in historical biogeography

A hypothesis of relationships of the families of Sciaroidea (Cecidomyiidae excluded) has been given by MATILE (1986,1990) (for a review of earlier hypotheses see MATILE, 1986 376-410), where the Sciaridae are considered the sister-group of the Mycetophilidae + Lygistorrhinidae (Fig 1) In this paper, a new hypothesis founded on a greater number of characters is proposed for the Sciaroidea

Source MNHN Paris

Most larvae of Sciaroidea are more or less narrowly linked to the carpophores of the higher fungi, either spinning a web under or close to the hymenium (all Diadocidiidae, certain Keroplatidae and Mycetophilidae), or living in the carpophore itself (some Ditomyiidae, all Bolitophilidae, most Mycetophilidae, some Sciaridae). They feed on spores only (sporophagy, fungivory *sensu lato*), on spores and hyphae, and perhaps in some cases on hyphae only. Some species live in rotten wood, where they feed on mycelia (some Ditomyiidae and Sciaridae). On the other hand, most Sciaridae live in the soil litter, were they are thought to be saprophagous. Some very few species of Sciaridae and Mycetophilidae are phytophagous, while many Keroplatidae and some Mycetophilidae are predaceous. Most Cecidomyiidae larvae are phytophagous, but there are predatory or fungivorous species.

A number of traits are more or less narrowly associated with larval feeding habits: secretion of silky threads to spin webs and or pupal cocoons, endobiosis or epibiosis and, in epibiosis, structure and composition of the web. PLACHTER (1979b) assumed that the three-dimensional web of certain Keroplatidae species was derived from a primitive, less complex web with a wide central band. ZAITSEV (1984) thought that the first step towards fungivory in Sciaroidea had been epibiont "grazers" feeding on mycelia covering wood, leaves, and other substrata. In a previous work, the larval morpho-ecology of the Sciaroidea was studied. The ancestral stock of the superfamily was presumed to be a eurybiont detritophilous larva in the sense of MAMAEV (1968, 1975), *e.g.* a fungivorous larva of soil, litter and rotting wood, without silk secretion and bearing well-developed antennae and body macrochaetae (MATILE, 1986). The groundplan of the Diadocidiidae-Keroplatidae clade and its sister-group has been inferred as having an endobiont larva, to be fungivorous and silk-producing, and to have a smooth and long body, with vestigial antennae, eyes and macrochaetae, however these provisional conclusions were not published in my 1990 monograph.

MATERIALS AND METHODS

The imaginal morphological data used in the phylogenetic hypotheses discussed here was provided mainly by material from the Collections of the Museum National d'Histoire naturelle, Paris. The remainder came from specimens loaned by many colleagues and institutions worldwide (*cf.* MATILE, 1990: 24-25, 637). I have thus been able to examine adults of representative species (most often at least the type-species) of practically every known genus of Sciaroidea, with the notable exception of the Sciaridae, where only the largest genera have been checked. Data on the Cecidomyiidae were mostly obtained from the literature.

Concerning the name of Sciaroidea versus Mycetophiloidea, the superfamily name Sciaroidea is founded on *Sciaraedes* Billberg, 1820, while Mycetophiloidea is based on *Mycetophilites* Newman, 1834 (SABROSKY, pers. com.). Although names of the family-group using the prefix Mycetophil- are many times more numerous than those founded on Sciar-, art. 36a (Principle of coordination for the family-group names) of the International Code of Zoological Nomenclature imposes the use of Sciaroidea, unfortunately resurrected from a long oblivion by MCALPINE *et al.* (1981).

As regards larvae, this paper is based on published works, especially those by MADWAR (1937), BRAUNS (1954a) and PLACHTER (1979a, 1979b, 1979c) for illustrations and observations, and by LASTOVKA (1972), ZAITSEV (1984) and MATILE (1986, 1990) for interpretation and homology. Personal observations, published or not, have been added - these have been accumulated over thirty years, in the field and in the laboratory, in tropical and temperate areas.

Concerning the polarization of characters, the analysis by MATILE (1990) has generally been followed; it bears mainly on Keroplatidae, but can easily be extended to the Sciaroidea. The matrices of characters have been treated by the Hennig86 program (FARRIS, 1988), with implicit enumeration ("ie" command), characters non-ordered ("cc -" command), and the evolution of the characters has been followed with the Clados program (NIXON, 1991). After phylogenetic hypotheses for the groups involved were obtained, it was possible to proceed to an optimization (FARRIS, 1970) of the different attributes on the cladograms. The seven attributes studied are identified and numbered in the text, the first three bear on the Bibionomorpha and Sciaroidea, the last four on Keroplatidae only.

Source: MNHN, Paris

PHYLOGENY

The few phylogenetic hypotheses expressed up to now on Sciaroidea have been manually obtained, except in OOSTERBROECK & COURTNEY'S analysis, which bears on all families of "Nematocera", and was done using the programs Paup and Hennig86 All hypotheses autapomorphic family traits excluded have taken few characters into consideration 4 IN WOOD & BORKENT's, 8 in OOSTERBROECK & COURTNEY's, 9 in MATILE's I have been able to gather a greater number of characters (30), either new or already available in the literature, which allows the reconstruction of a new cladogram (although yet provisional) of the Sciaroidea

Appendix I refers to these characters and their matrix, and gives the references to their polarization when already published The outgroups chosen are the Bibionidae (genus *Bibio* Geoffroy) and the Pachyneuridae (genus *Cramptonomyia* Alexander), both basal to the Bibionomorpha in WOOD & BORKENT's and OOSTERBROEK & COURTNEY's cladograms *Cramptonomyia* (here Pachyneuridae) and *Hesperinus* Walker (Bibionidae), *Plecia* Meigen and allied genera (Bibionidae) are sometimes considered as of family rank The position of the Pachyneuridae is debatable For example the family has been placed with Axymiidae and others in an infraorder Axymyiomorpha (WOOD & BORKENT, 1989) or a superfamily Pachyneuroidea (KRZEMINSKA *et al* , 1993) For other authors Axymyiidae stand by themselves in Axymyioidea (SHCHERBAKOV *et al* , 1995), or the group Cramptonomyiformia is proposed (AMORIM, 1992), etc (see review in AMORIM, 1992) The reader is therefore reminded that I do not purport to give here a new hypothesis on the phylogeny of the Bibionomorpha the introduction in the cladogram of a genus each of Bibionidae and Pachyneuridae derives from the necessity of choosing at least two outgroups The Cecidomyiidae have been taken into account because of their presumed sister-group relationship with the Sciaridae rather than the rest of the Sciaroidea

Autapomorphies of the families have been excluded from the analysis, as well as those of the Bibionomorpha We have not used OOSTERBROECK & COURTNEY's character 90 (absence of sperm pump), because it is present at least in some Keroplatidae (MATILE, 1990) Their character 72 (anterior veins concentrated along costal margin) has also been eliminated because this costalization is common in Keroplatidae and Mycetophilidae

Phylogenetic analysis in Hennig86 gave only one most parsimonious tree, length 44, CI 0 70 and RI 0 76 (Fig 2) This tree shows the Cecidomyiidae as the sister-group of the Ditomyiidae+ group (terminology of AMORIM, 1982) as advocated, although with some doubts, by HENNIG (1954, 1970, 1973) The structure of the Ditomyiidae+ group agrees with the cladogram of the "Mycetophiloidea" given by MATILE (1990), and there seems no necessity to retain the Cecidomyiidae as a superfamily by themselves The cladogram agrees also with the phylochronogram given by SHCHERBAKOV *et al.* (1995) as regards the recent families, and especially the sister-group relationship of Cecidomyiidae and Sciaroidea

It is to be noted that several characters are yet unknown in some terminal taxa, especially number 14 (loss of dorsal transverse connective in larval tracheal system), 29 (chromosomic elimination) and 30 (loss of central sperm microtubule) The cladogram implies that character 14 has appeared by homoplasy in Sciaridae and Cecidomyiidae, and that characters 29 and 30 are basal for the Bibionomorpha, with a reversal in the clade Mycetophilidae-[Lygistorrhinidae]

The Keroplatidae have been divided into three subfamilies, Arachnocampinae, Macrocerinae and Keroplatinae (MATILE, 1981a) In addition, two tribes have been recognized in

Source MNHN Paris

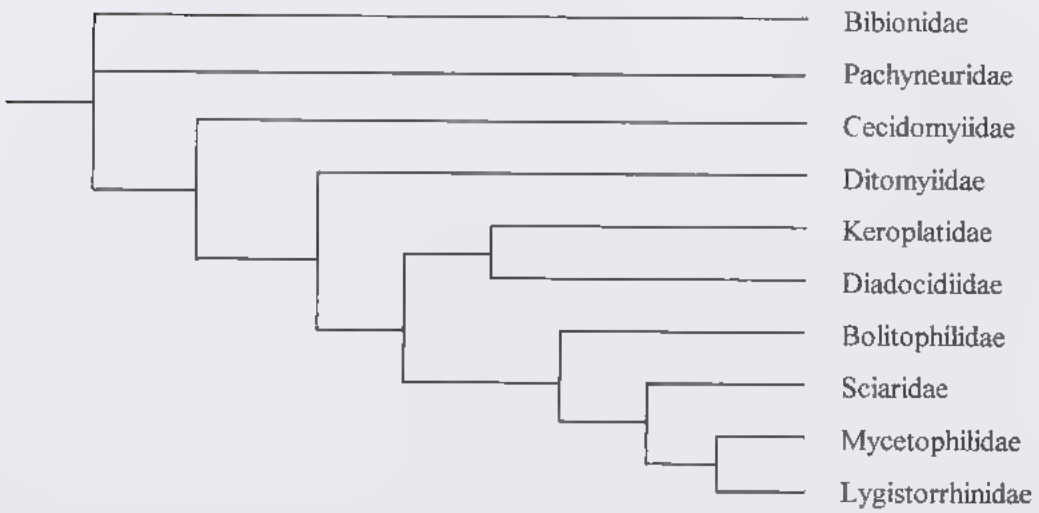

FIG. 2. — Phylogenetic relationships of the Sciaroidea. Hennig86, 30 characters, unordered, "ie" command. Length: 44, CI = 0.70, RI = 0.76.

the last two subfamilies Macrocerini and Robsonomyini in Macrocerinae, and in Keroplatinae Keroplatini and Orfeliini (MATILE, 1990). A phylogenetic analysis of these three families has been given in MATILE, 1990, on the basis of 40 larval, pupal and imaginal characters. This analysis was hand-treated, and I present here a quantitative analysis bearing on 45 characters (without the two tribes of Macrocerinae, the larvae of only *Macrocera* being known). The outgroup chosen is the family Ditomyiidae. The characters, polarized after MATILE, 1990, and their matrix are given in Appendix II. The treatment of the matrix by Hennig86 gave only one tree, length 45, with high indices : CI = 0.97, RI = 0.93 (Fig. 3). The tree has the same structure than that published in 1990.

Arachnocampinae are monogeneric and only the larvae of *Macrocera* Meigen are known in Macrocerinae. We possess larval data for only 15 out of the 73 present genera of Keroplatinae (in fact 17, but for two genera, *Platyroptilon* Westwood and *Neoceroplatus* Edwards, the data are incomplete). This lack of knowledge is not surprising since one of the attributes of many Keroplatidae is precisely cryptobiosis. To leave out unnecessary noise (and pending a generic revision of the Orfeliini, an enterprise which should take several years), the analysis has been pursued only on the following 15 genera : 5 genera of Keroplatini (*Cerotelion* Fabricius, *Heteropterna* Skuse, *Keroplatus* Bosc, *Mallochimus* Edwards, *Tergostylus* Matile) and 10 genera of *Orfeliini* [*Neoditomyia* Lane & Stürm, "*Neoplatyura*" *fultoni* (Shaw), which should probably be given a genus by itself, *Orfelia* Costa, *Planarivora* Hickman, *Platyceridion* Tollet, *Platyura* Meigen, *Proceroplatus* Edwards, *Truplaya* Edwards, *Urytalpa* Edwards, *Xenoplatyura* Malloch].

Appendix III lists the 14 characters (generic autapomorphies excluded) used in the analysis of the Keroplatini with known larvae and their matrix. *Arachnocampa* is used as the outgroup.

The matrix treated by Hennig86 gave only one tree, length 19, CI = 84, RI = 86. The tree (Fig. 4) is not different from the one which can be deduced from the general cladogram of the Keroplatini proposed in MATILE (1990).

Source : MNHN, Paris

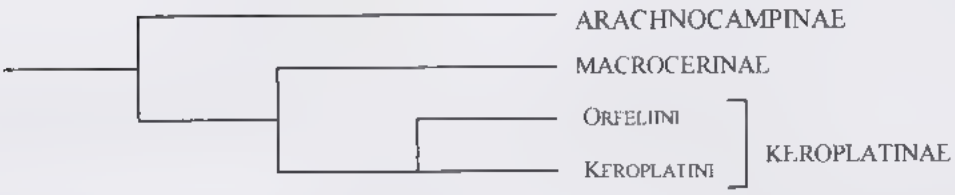

FIG 3 Phylogenetic relationships among subfamilies and tribes of Keroplatidae Hennig 86, "ie" command, 45 characters Length 45, CI = 0 97, RI = 0 93

FIG 4 Phylogenetic relationships among five genera of Keroplatini Hennig 86, 14 characters "ie" command, unordered. Length 19, CI = 0 84, RI = 0 86

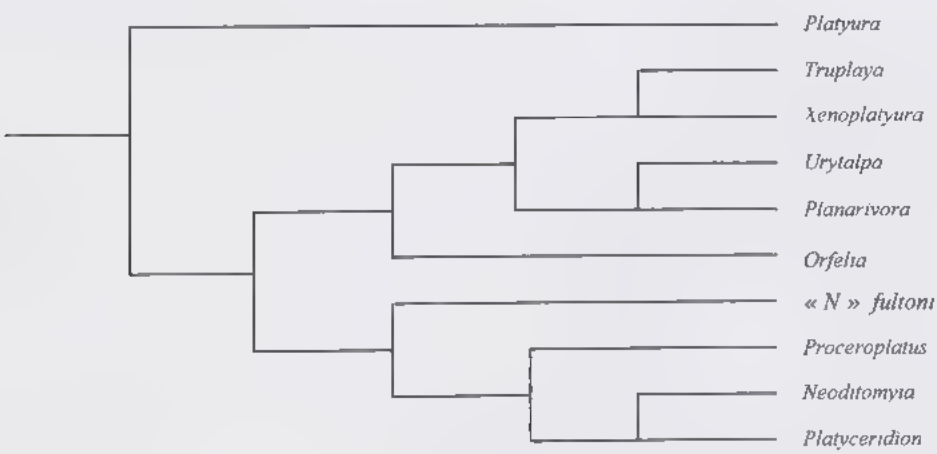

FIG 5 Phylogenetic relationships among ten genera of Orfeliini Hennig86, 23 characters, "ie" command, unordered Length 42, CI = 0 57, RI = 0 66

Twenty-seven characters (generic autapomorphies excluded) have been used in the phylogenetic analysis of the 10 Orfeliini genera with known larvae, they are listed in Appendix IV, with the corresponding matrix *Arachnocampa* has been chosen as the outgroup Hennig86 gave only one tree, length 42, CI = 0 57, RI = 0 66 (Fig. 5)

Source MNHN Paris

The studied Orfeliini fall readily in two sister-clades, *Orfelia*+ and "*N.*" *fultoni*+, with *Platyura* being their sister-group.

ATTRIBUTES

Four attributes of the larvae of Keroplatidae will be studied. In order to study the evolution of the food-linked traits of this family, it is first necessary to consider them at the superfamily level, as well as in the outgroups chosen.

A study of the Bibionomorpha as a whole leads to the consideration of three attributes, which will be useful in the following analyses:

Endobiosis vs epibiosis. Endobiont larvae live inside their source of food, epibiont larvae live outside their source of food.

Fungivory sensu lato *vs various other preferences*. Fungivorous larvae feed on hyphae and/or spores inside the fruiting bodies of higher fungi, or on mycelium in rotten wood or litter, or feed exclusively on spores falling from the hymenium of Polyporaceae. These three categories belong to fungivory *s.l.*

Silk secretion. Many larvae secrete silk for various purposes, and at least for the building of the last instar cocoon, in which the larva will pupate. Others have no silk secretion and naked pupae.

The attributes of the Keroplatidae to be studied are as following.

Predation vs sporophagy. These terms are self-explanatory, taking into account that the "fungivory" of Keroplatidae is restricted to spores escaping from the hymenium of bracket-fungi.

Net-like web vs sheet-like web. Some keroplatid larvae spin a diffuse, three-dimensional web bearing fishing lines (Figs 6, 8, 10). Others secrete a wide, mostly two-dimensional web (Fig. 7).

pH of web. The webs spun by the larvae bear drops of an acid labial fluid, according to species, the pH stands between 1 and 2.7 or, in other cases, is higher than 3.

Cryptobiosis vs optobiosis. These new terms are coined for epibiont larvae living in hidden, obscure and water saturated cavities, such as under stones or rotting trunks, in phytotelma, etc. (cryptobionts). In contrast, other epibiont larvae live more in the open, on the walls of caves, under bracket-fungi, under overhanging cliffs or stream banks, under leaves, etc. (optobionts).

Bibionid larvae are mostly scavengers or plant-feeding, living in the soil on roots, grasses and decaying plant material (HARDY, 1981, and references therein). Larvae of Pachyneuridae are associated with rotten wood (VOCKEROTH, 1974, KRIVOSHEINA & MAMAEV, 1988), whether they are true xylophagous insects or feed on mycelia inside the wood has not been ascertained.

Most Cecidomyiidae larvae are plant-feeding and gall-forming, but the more basal are fungivorous (MAMAEV, 1975, GAGNE, 1986). There are also some endoparasites and some predatory species on insects and mites, a habit that evolved separately several times according to GAGNÉ (this author has also noted the anatomo-morphological exaptations to plant-feeding shown by the "ancestral" fungus-feeding species: sucking mouthparts, extra-intestinal digestion and spatula). Cecidomyiidae larvae are epibionts or endobionts and most secrete silk at least for the cocoon in which they will pupate.

Source: MNHN, Paris

In the Ditomyiidae, larvae of *Ditomyia* Winnertz feed on hard bracket-fungi such as *Coriolus versicolor* and others, and those of *Asioditomyia japonica* (Sasakawa) have been found in *Lenzites betulina*. *Symmerus* Walker and *Australosymmerus* Freeman are said to be xylophagous (MADWAR, 1937, MUNROE, 1974), however CHANDLER (1993) cites *Symmerus annulatus* (Meigen) reared from a hard ascomycete fungus, *Hypoxylon rubiginosum*. The term "frass" referred to by MUNROE for the substance left in their galleries by larvae of *S. coqulus* Garrett is ambiguous, since it can apply to either larval excrement or saw dust left by xylophagous animals. It is very possible that all Ditomyiidae larvae found in rotten wood feed on the mycelia that it contains. There is no pupal cocoon, the pupation takes place in the substratum.

Feeding habits of the larvae of Diadocidiidae have been uncertain for a long time (BRAUNS, 1954a). I have often observed them under rotten wood invaded either by mycelia or by encrusting Polyporaceae. In specimens killed in fixative fluid, spores desegregating progressively towards the rear of body were observed, and the discovery of *Diadocidia ferruginosa* (Meigen) on *Peniophora* sp. (CHANDLER, 1993) confirms the fungivory of the family. Diadocidiid larvae are epibionts and spin a silky tube and a pupal cocoon.

Bolitophilidae have strictly endobiont fungicolous larvae and breed mostly in Strophariaceae, Cortinariaceae, Polyporaceae and Boletaceae (HUTSON *et al.*, 1980). They spin neither web nor pupal cocoon.

Keroplatidae comprise either predators or sporophagous larvae, rarely species showing a mixed diet (*cf.* MATILE, 1986, 1990). *Cerotelion*, a normally sporophagous species, has been recorded once as feeding on a recently dead larva, and once as cannibalistic (a pupa of its own species) (MANSBRIDGE, 1933). However, these observations on *Cerotelion* have been made in breeding jars where spores can get scarce, or disappear. On the other hand, first instar larvae of *Macrocera nobilis* Johnson are scavengers, and the following instars are predators (PECK & RUSSEL, 1976). All larvae of Keroplatidae spin webs and are epibionts, except those of the genus *Planarivora* Hickman, or at least of their Tasmanian representative, *Planarivora insignis* Hickman, which is an endoparasitoid of land planarians, but nonetheless spins a pupal cocoon after emerging from its dead host (HICKMAN, 1965).

The food preferences of mycetophilid larvae are very diverse, ranging from predation, with epibiont web-spinning species, to phytophagy (but exclusively linked with liverworts – in two clades Mycomyinae and Gnoristinae), through true fungivory, the most common diet. Most known larvae live in a hygroscopic web, except in subfamily Mycetophilinae. Almost without exception there is a pupal cocoon (*Speolepta* Edwards, no cocoon, some Mycetophilini genera, a pupal case).

Sciaridae comprise some true fungivorous, phytophagous, coprophagous and xylophagous species, but mostly they are litter forms, where they probably feed on mycelia (STEFFAN, 1981). They spin webs and pupal cocoons.

The feeding habits of the studied families of Bibionomorpha are summarized in Table I. The term "xylophagous" has been put between brackets because it is not known with certainty if larvae of Pachyneuridae, some Ditomyiidae and some Sciaridae are really wood-eating, or rather feed on mycelia in rotten wood.

Source: MNHN, Paris

TABLE 1 Food preferences of the larvae of Bibionomorpha. Legends: end/epi = endo- or epibionts; silk = silk secretion, [xylo] = xylophagous, pred. = predators, fungic = fungicolous; creo-fung. = creo-fungicolous, phytoph. = phytophagous; saproph. = saprophagous, parasit = parasitoids, liverw. = liverworts.

Families	Food								
	end/epi	silk	[xylo.]	pred.	fungic.	creo-f.	phytoph.	saproph.	parasit.
Bibionidae	end.	0	0	0	0	0	+	+	0
Pachyneuridae	end.	0	+	0	?	0	0	0	0
Ditomyiidae	end.	0	+	0	+	0	0	0	0
Diadocidiidae	epi.	+	0	0	+	0	0	0	0
Keroplatidae	epi.	+	0	+	+	+	0	0	+
Bolitophilidae	end.	0	0	0	+	0	0	0	0
Mycetophilidae	end./epi.	+	0	+	+	0	liverw.	0	0
Sciaridae	end./epi.	+	+	0	+	0	0	+	0
Cecidomyiidae	end./epi.	+	0	+	+	+	+	0	+

Larvae of Keroplatidae have very diverse feeding habits. Members of the Arachnocampinae (monogeneric), and *Macrocera* in Macrocerinae (first instars of all other genera are unknown) are predators of insects and other small invertebrates, first instar larvae of at least one species of *Macrocera* being scavengers. In the Keroplatinae, known Orfeliini are predators (*Neoditomyia*, "*Neoplatyura*" *fultoni*, *Orfelia*, *Platyceridion*, *Platyura*, *Proceroplatus*, *Truplaya*, *Xenoplatyura*), except *Planarivora*, a parasitoid, and *Urytalpa*, feeding habit unknown (but see below). Three genera have been discovered recently, that even attack ants. *Truplaya* (KOVAC & MATILE, in press), *Proceroplatus* (ALELLO & JOLIVET, in press, MATILE, in press) and *Platyceridion* (CHANDLER & MATILE, in prep.). All known Keroplatini are spore-feeders (*Cerotelion*, *Heteropterna*, *Keroplatus*, *Mallochinus*, *Tergostylus*), with *Cerotelion*, as already noted, occasionally able to eat dead or immobile prey, at least in captivity. Two other species of Keroplatini, belonging to genera *Platyroptilon* and *Neoceroplatus*, have been found in connection with rotten wood invaded by mycelia or with bracket-fungi (DURET, 1974, MATILE, 1982), and are very probably spore-feeders.

Larvae of *Arachnocampa* and *Neoditomyia* are found in natural and artificial caves and tunnels, but also in more open conditions such as under leaves (PUGSLEY, 1984, MATILE, 1990, 1994, STURM, 1973, JACKSON, 1974), while Keroplatinae Keroplatini live mostly under bracket-fungi, either on standing trees or fallen trunks and branches (MATILE, 1990, and references therein). All of these are therefore optobionts. *Macrocera* larvae are cryptobionts, except in cavernicolous conditions (MATILE, 1990, 1994, and references therein). All known Orfeliini except *Neoditomyia* (tropical caves and forests) are also cryptobionts, living in deeply burrowed cavities under stones or fallen trunks, in bamboo internodes or domatia of ant-plants (PLACHTER, 1979a, 1979b, MATILE, 1990, and references therein, JOLIVET, 1996, KOVAC & MATILE, in press, ALELLO & JOLIVET, in press, MATILE, in press, CHANDLER & MATILE, in prep.).

The webs of keroplatid larvae have three more or less distinct parts: a central tube in which the larva moves when active; a shelter web, usually hidden in some secondary cavity of the substratum; and a feeding web. For predators, the feeding web comprises a more or less dense

Source: MNHN, Paris

TABLE 2. — Food preference and other attributes of larvae of Keroplatidae (pH data from PLACHTER, 1979a)

Genera	food	pH of web	net shape	cryptobiosis
Arachnocampa	predator	"very acid"	net	0
Macrocera	predator	2,4 - 2,7	net	+
Platyura	predator	1,3 - 1,6	net	+
Truplaya	predator	?	net	+
Xenoplatyura	predator	1-2	net	+
Urytalpa	?	3,6 - 4,0	net/sheet	+
Planarivora	parasitoid	?	pupa only	+
Orfelia	predator	1,3 - 1,6	net	+
"*N.*" *fultoni*	predator	?	net	+
Proceroplatus	predator	?	net	+
Neoditomyia	predator	?	net	0
Platyceridion	predator	?	net	+
Cerotelion	creo-sporo.	1,3 - 1,6	net/sheet	0
Mallochinus	?	?	?	0
Keroplatus	sporoph.	3,0 - 3,4	sheet	0
Tergostylus	sporoph	not toxic	sheet	0
Heteropterna	sporoph	not toxic	sheet	0

system of crossed lines, from which drop "fishing lines", short or long according to the space available Shelter and feeding webs bear numerous droplets of saliva containing oxalic acid, sometimes in very high concentration (BUSTON in MANSBRIDGE, 1933, PLACHTER, 1979a) All are individual webs, but can be re-used by later generations in some species of cave-dwelling *Macrocera* (PECK & RUSSEL, 1976) These predator systems will be called "net-webs" All have a pH of 2 7 or less In the webs of spore eaters, the fishing net is replaced by a wide mucous film which covers the hymenium, and is often collective, sheltering larvae of several instars these will be called sheet-webs The saliva of at least some of these sporophagous species also contains oxalic acid *Keroplatus* has a pH of 3 or more, but *Cerotelion* is highly acid, and has a web (Fig 9) intermediate between the net type and the sheet type (crossed lines, but no fishing lines) Small invertebrates introduced in webs of *Tergostylus* and *Heteropterna* have shown no particular reactions (as with *Keroplatus*), and it may be inferred that their acidity is also weak The sheet-webs of *Keroplatus tipuloides* Bosc can be re-used by later generations (MATILE, pers obs) All these data are summarized in Table 2

In summary, examination of the table shows that when known, the pH is always correlated with the type of the web 2 7 or less for net-webs (predators), 3 or more for sheet-webs (spore-feeders) *Cerotelion*, which has an intermediate web and, although spore-feeding, can show predatory behavior, at least in laboratory conditions, is an exception It is easy to deduce from this that *Urytalpa* must be a spore-feeder The only species whose larva is known, *U. ochracea* (Meigen), was observed "practically swimming" in its sheet-like web (PLACHTER, 1979a), a behavior which does not imply an ambushing predator The web of *U. ochracea* is not exactly a sheet the central thread is not wide and ribbon-like, but instead there are triangular

Source MNHN Paris

accumulations of fluid secretion at its emplacement, where most of the fluid secretion concentrates (PLACHTER, 1979a) Regarding predation, one can add that one species each of *Platyceridion, Proceroplatus* and *Truplaya* are ant-eating

MAPPING THE ATTRIBUTES ON THE CLADOGRAMS

With the phylogenetic hypotheses for the groups involved, it is now possible to proceed to feeding habit optimization, first at the suprafamilial level, then for the Keroplatidae At the suprafamilial level, we shall study three attributes, fungivory in its broad sense (as opposed to various other diets), endobiosis or epibiosis, and silk secretion For the Keroplatidae, the four attributes studied are those indicated in Table 2, *e g* predation vs sporophagy (*Urytalpa* being considered a spore-feeder), highly acid pH vs less acid pH, net-web vs sheet-web, and optobiosis vs cryptobiosis

Fungivory The optimized cladogram (Fig 11) suggests that the Ditomyiidae are truly primarily fungivorous (two lines have been drawn, since fungivory of several genera is not ascertained) and that fungivory *sensu lato* is most probably ancestral to the Sciaroidea + Cecidomyiidae lineage

Epibiosis All terminal taxa of the cladogram are endobionts except in the clade Keroplatidae-Diadocidiidae, where epibiosis may be assumed ancestral (Fig. 11) This way of life appeared independently in certain Mycetophilidae and Cecidomyiidae

Silk secretion For silk secretion, the optimized cladogram (Fig 12) shows that its acquisition is ancestral for the Sciaroidea A loss in the Ditomyiidae and one in Bolitophilidae, is more parsimonious (two steps) than an independent appearance in Keroplatidae-Diadocidiidae, in the Sciaridae+ clade and in the Cecidomyiidae (three steps)

Food preferences The optimized cladogram on food preference (Fig 13) shows that the ancestral condition of the Keroplatidae is predation, as hypothesized by ZAITSEV (1983) and MATILE (1986) Sporophagy has appeared twice, once in *Urytalpa* and once in the Keroplatini clade, but is not yet stabilized in *Cerotelion*, and perhaps *Mallochinus* (the only indication we have on the larval biology of this genus is that its habits correspond to that of *Keroplatus* as described by DUFOUR in 1839) Sporophagy is therefore a new specialization from predation LASTOVKA'S hypothesis (1972), according to which predation probably evolved from sporophagy, is thus refuted

Net-like web vs sheet-like web, and pH of web Table 2 shows that these characters are correlated with the food preference of the larvae Thus net-webs with a high acidity are ancestral

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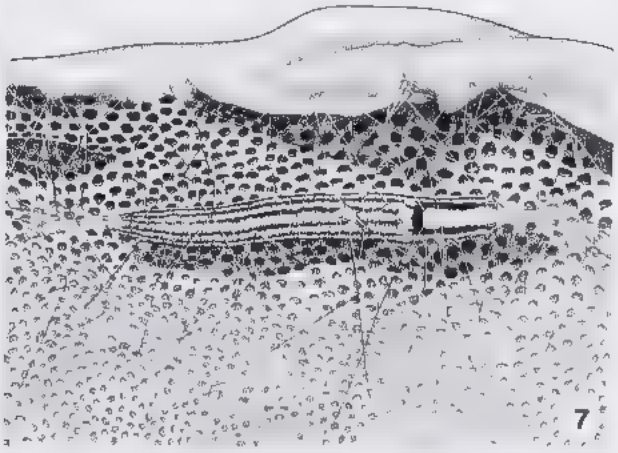
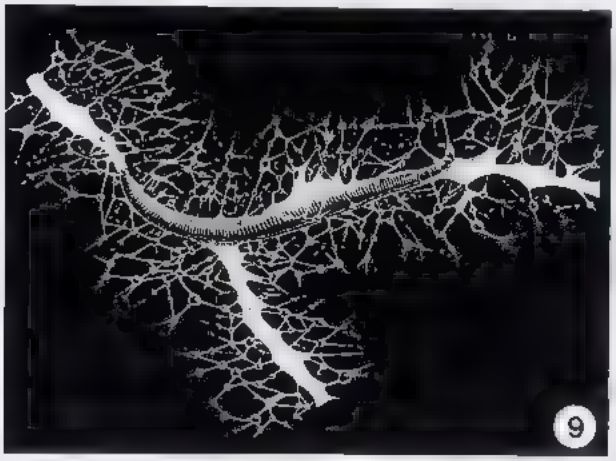
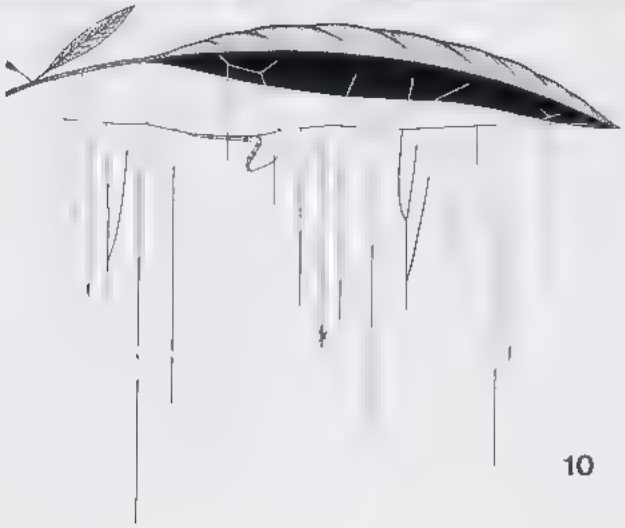
Figs 6-10 Larvae of Keroplatidae in their webs **6**: *Arachnocampa luminosa* (Skuse) (New Zealand) in a crevice of a cave wall with suspending lines central tube and long fishing lines **7**: *Heteropterna chazeaui* Matile (New Caledonia) on the underside of a bracket-fungus the larva hangs in a translucent sheet only the central tube and attaching lines are visible **8**: *Macrocera fasciata* Meigen Europe) hanging from the ceiling of a quarry, with central thread and net web with short fishing lines **9** *Cerotelion lineatum* Fabricius) (Europe) on underside of a bracket-fungus there are two central tubes, dense lines and no fishing lines **10**. *Neoditomyia aerospicator* (Jackson) (Central America) under a leaf in tropical rain-forest, with suspending threads, central tube and fishing lines Length of mature larvae *A luminosa*, 3-4 cm, *M fasciata* *N aerospicator*, *C lineatum* 2,5-3 cm *H chazeaui*, 2-2,5 cm Fig from MATILE, 1990 except 10, a combination of a photograph of *N aerospicator* by JACKSON (1974), and a sketch of attitude of larva of *N andina* LANE in STÜRM (1973)

Source MNHN, Paris







Source MNHN, Paris

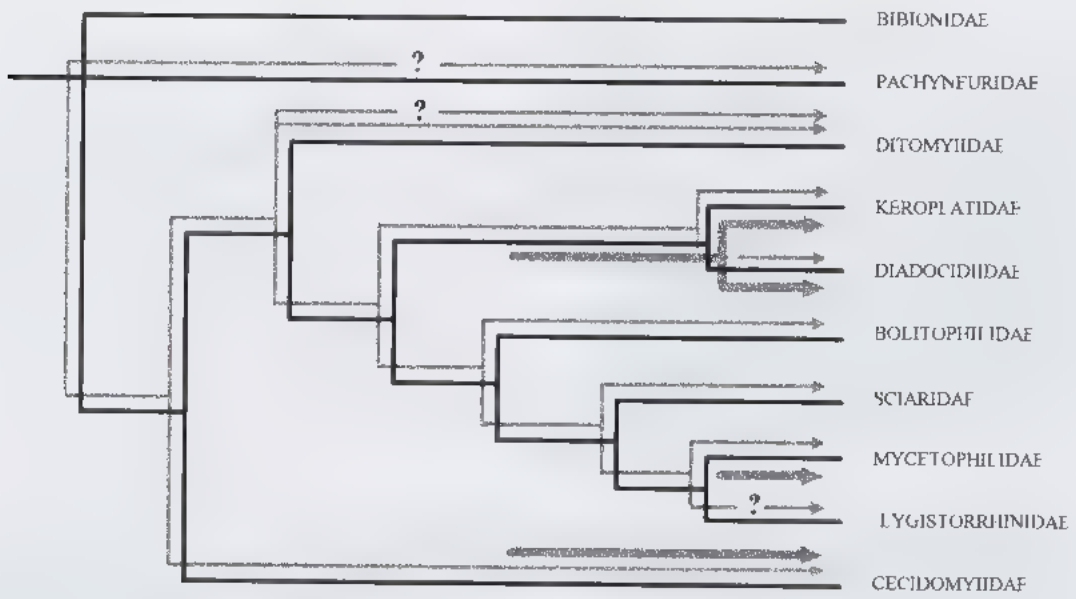


FIG. 11 Evolution of fungivory *s. l.* (thin grey lines) and appearance of epibiosis (thick grey lines) in the Bibionomorpha

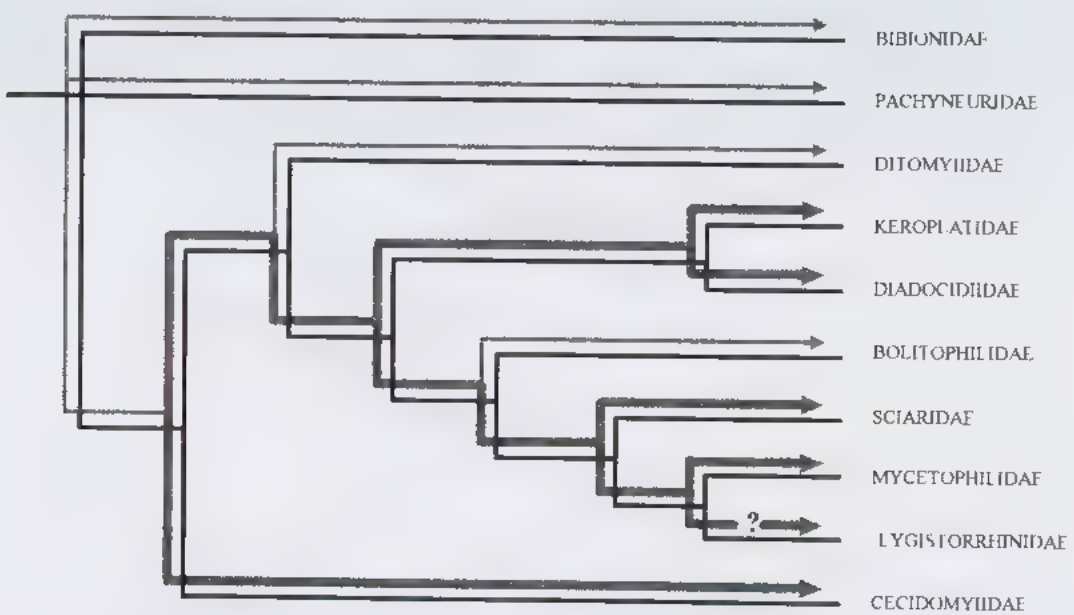


FIG. 12 — Absence (thin grey lines) or presence (thick grey lines) of silk secretion in the Bibionomorpha

Source: MNHN, Paris

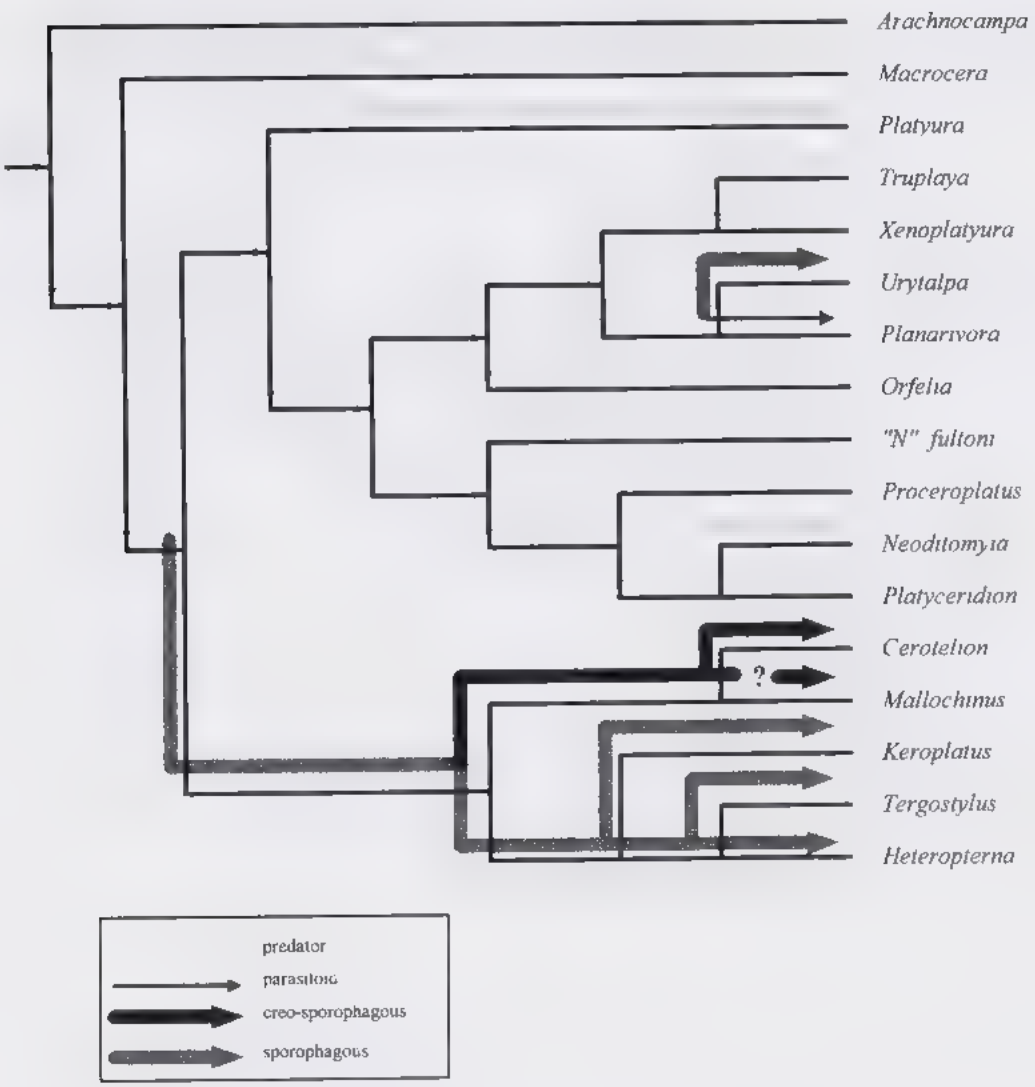


Fig. 13. — Food preferences of the known larvae of Keroplatidae.

for the Keroplatidae, while sheet-webs with low acidity are derived These last features are characteristic of the Keroplatini clade and of the genus *Urytalpa*, and have appeared at least twice PLACHTER's (1979c) hypothesis of plesiomorphy of the sheet-web is therefore refuted

Cryptobiosis and optobiosis As regards cryptobiosis and optobiosis, the superposition of these attributes on the cladogram (Fig 14) gives two equally parsimonious scenarios (3 steps) If cryptobiosis is ancestral, tolerance to light must have appeared at least three times in *Arachnocampa*, *Neoditomyia* and the Keroplatini On the other hand, if optobiosis is ancestral, then cryptobiosis must have appeared independently once in *Macrocera* and once in the Orfelini clade, with a reversal in *Neoditomyia*

Source MNHN Paris

However, endobiosis is the ancestral condition of the Bibionomorpha, as shown on Fig 11 Bibionid, pachyneurid and ditomyiid larvae live in closed, obscure and humid galleries, and we may therefore infer that cryptobiosis is the ancestral condition of keroplatids

THE TEMPORAL DIMENSION

The study of the evolution of the attributes of the Bibionomorpha, and especially of the Keroplatidae, can be refined by taking into account the temporal dimension The oldest Diptera are known from the Trias of Australia, North America and Europe (EVENHUIS, 1994, SHCHERBAKOV *et al*, 1996, and references in both works) As regards the recent families of Bibionomorpha, the earliest fossils are known from the Upper Triassic for the Bibionidae, the lower Jurassic for the Mycetophilidae, the Upper Jurassic for the Pachyneuridae, the Upper Jurassic/Lower Cretaceous for the Cecidomyiidae, the Lower Cretaceous for the Keroplatidae and the Sciaridae, the Eocene for the Ditomyiidae, and the Eocene/Oligocene for the Diadocidiidae and the Lygistorrhinidae (EVENHUIS, 1994)

The fossil data, mapped on the cladogram, and the principle of equal age of sister groups (Fig 15), indicate that all the present families had appeared as such at least by the beginning of the Cretaceous, and more probably by the Upper Jurassic

The optimization of silk secretion and predation on the cladogram is given in Fig 16 Silk secretion must have appeared in the Sciaroidea clade at least by the Upper Jurassic Its disappearance in Ditomyiidae must be at least pre-Eocene, from which the extant genus *Australosymmerus* Freeman is known For Bolitophilidae, *Bolitophila* Meigen is known from Eocene-Oligocene, and the mesozoic fossils belong to the extinct genus *Mangas* Kovalev (perhaps not a Bolitophilidae at all), the larval biology of which is of course unknown, the loss of silk secretion cannot therefore be dated other than pre-Oligocene

Regarding predation (Fig 16), no data can be obtained from the optimized cladogram unless a further phylogenetic analysis is conducted for the three families in which it appeared independently

Fungivory and epibiosis are mapped on figure 17 Fungivory, ancestral to the Sciaroidea, must have appeared at least by the Upper Jurassic Epibiosis in the clade Diadocidiidae-Keroplatidae should be dated from the Lower Cretaceous, but its acquisition in Mycetophilidae and Cecidomyiidae cannot be dated without a further phylogenetic analysis of these families.

Fig 18 combines the palaeontological and biogeographical data on the cladogram of the Keroplatidae The two fossils appended to the *Macrocera* lineage represent two genera belonging to Macrocerinae, but of uncertain position inside the subfamily These are *Schlueterimyia cenomanica* Matile, from the Upper Cretaceous, and an undescribed genus from the Lower Cretaceous (GRIMALDI, 1990) The biogeographical datings can be found in MATILE (1990), except for *Planarivora* This genus has a southern transatlantic distribution New-Zealand and South America – and thus can be dated at the latest from the Upper Cretaceous (MATILE, 1981b).

The optimized chronocladogram (Fig 19) shows that the various food and web specializations of the family must have appeared at least by the end of the Lower Cretaceous, with the exception of the parasitoid habit, which dates from the Upper Cretaceous (assuming that the life-story of the neotropical species of *Planarivora* is the same as that of the Tasmanian

Source MNHN Paris

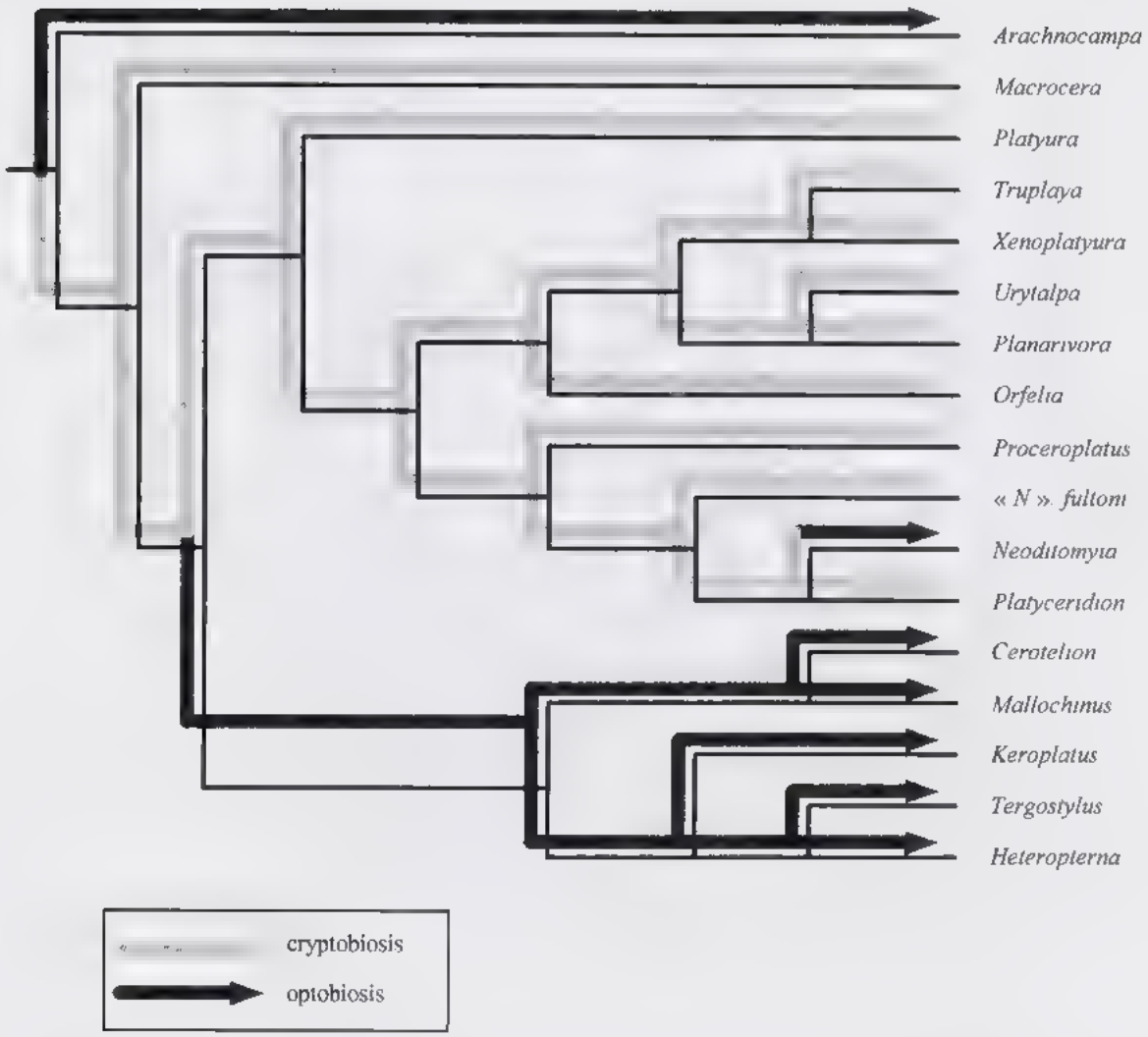


Fig 14 · Distribution of cryptobiosis and optobiosis in the Keroplatidae

species), and the independently acquired sporophagy of its sister-group *Urytalpa*, which may be presumed to be of the same age

In the same way, the cladogram of the figure 20 demonstrates the origin of cryptobiosis in the Lower Cretaceous, and of optobiosis in Keroplatini at least at the end of the same period

CONCLUSION

The optimization and dating of the cladograms allow inference of the ancestral larval state of the Bibionomorpha as an endobiont, as is the case for Sciaroidea, even if they contain numerous epibionts, which thus should have appeared more recently Fungivory is another

Source MNHN, Paris

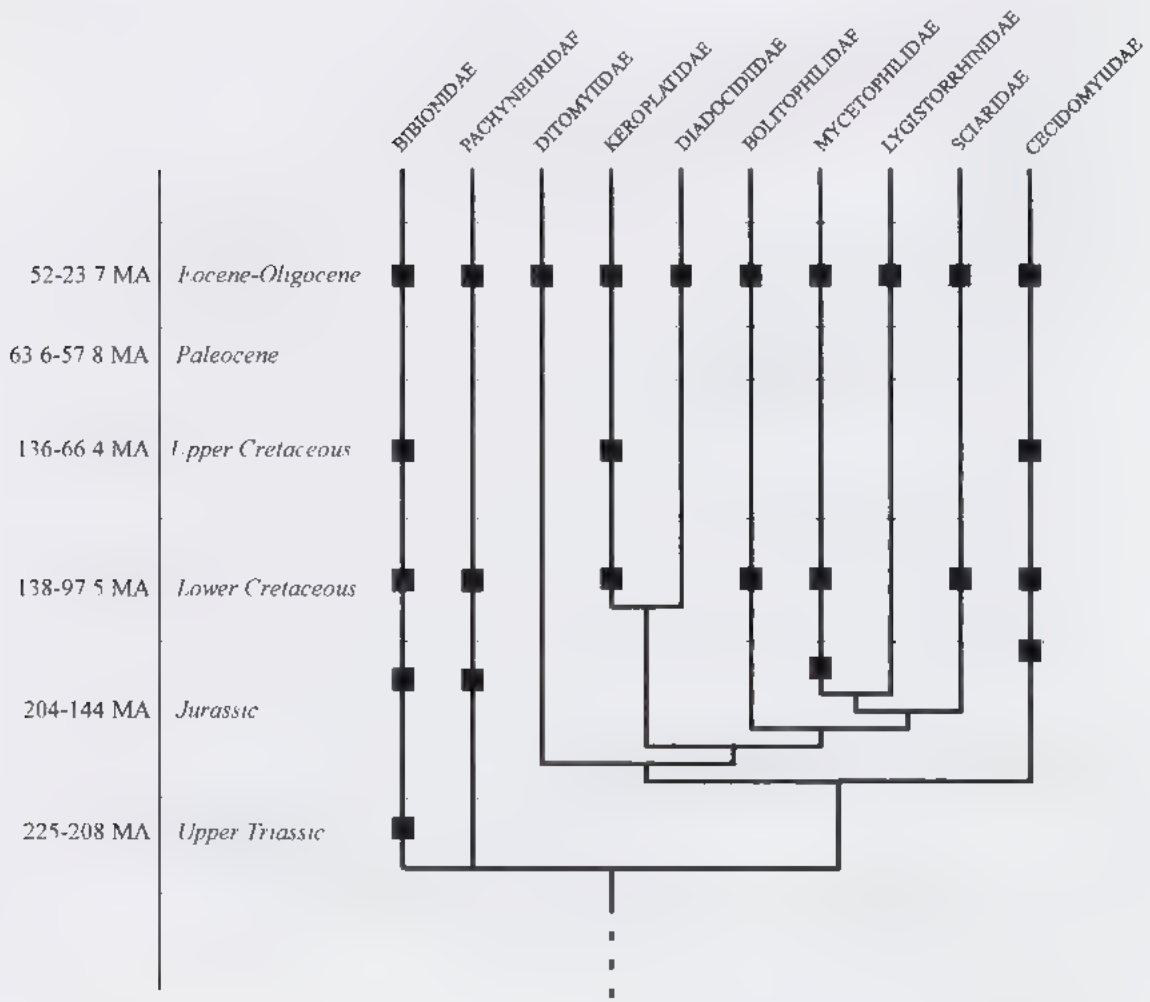


Fig 15 Pre-Miocene fossil data (black square) available on the taxa studied in this paper BIB – Bibionidae, PAC – Pachyneuridae, DIT – Ditomyiidae DIA – Diadocidiidae, KER – Keroplatidae, BOL – Bolitophilidae, MYC – Mycetophilidae, LYG – Lygistorrhinidae, SCI Sciaridae, CEC – Cecidomyiidae Geologic time scale after EVENHUIS (1994) For graphical reasons, no distinction has been made for Lower and Upper Jurassic

ancestral trait of Sciaroidea, as supposed by most authors ZAITSEV 's (1984) hypothesis of an epibiont ancestral larva for the fungicolous clades is thus refuted The endobiosis and fungivory of the ancestral Sciaroidea is corroborated by their larval morphology The larvae of this superfamily are indeed deprived of the anatomical tools necessary for predation their antennae are most often reduced to a cupule with a few sensillae, their organs of vision are rudimentary, and they have no well-developed sensorial macrochaetae This kind of morphology is not that of a predator, but of an animal living in the middle of an important amount of food, as noted by

Source MNHN Paris

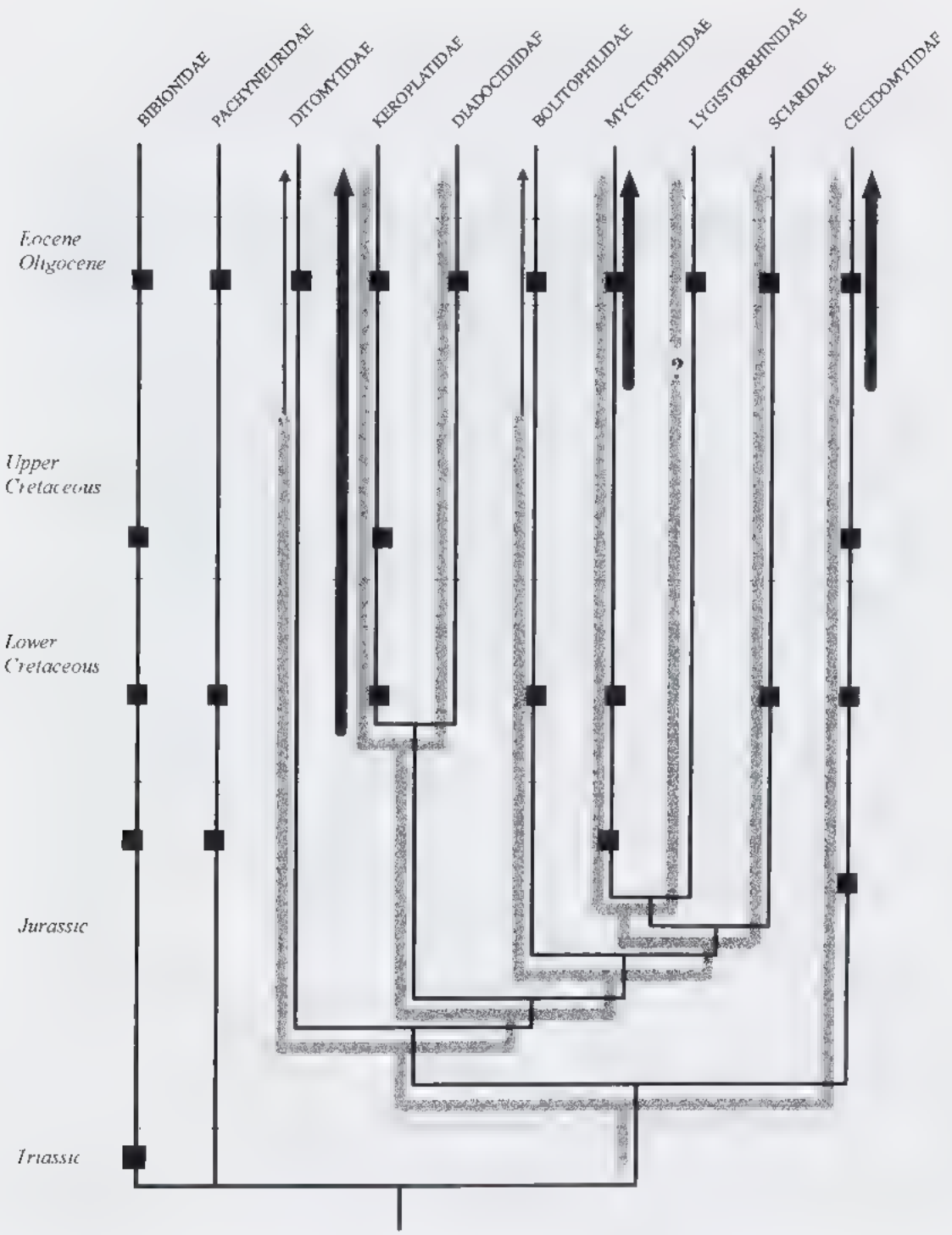


Fig 16 — Temporal evolution of silk secretion (thick light grey lines) and predation (thick dark grey lines) in the Bibionomorpha Black squares pre-Miocene fossil data

Source MNHN Paris

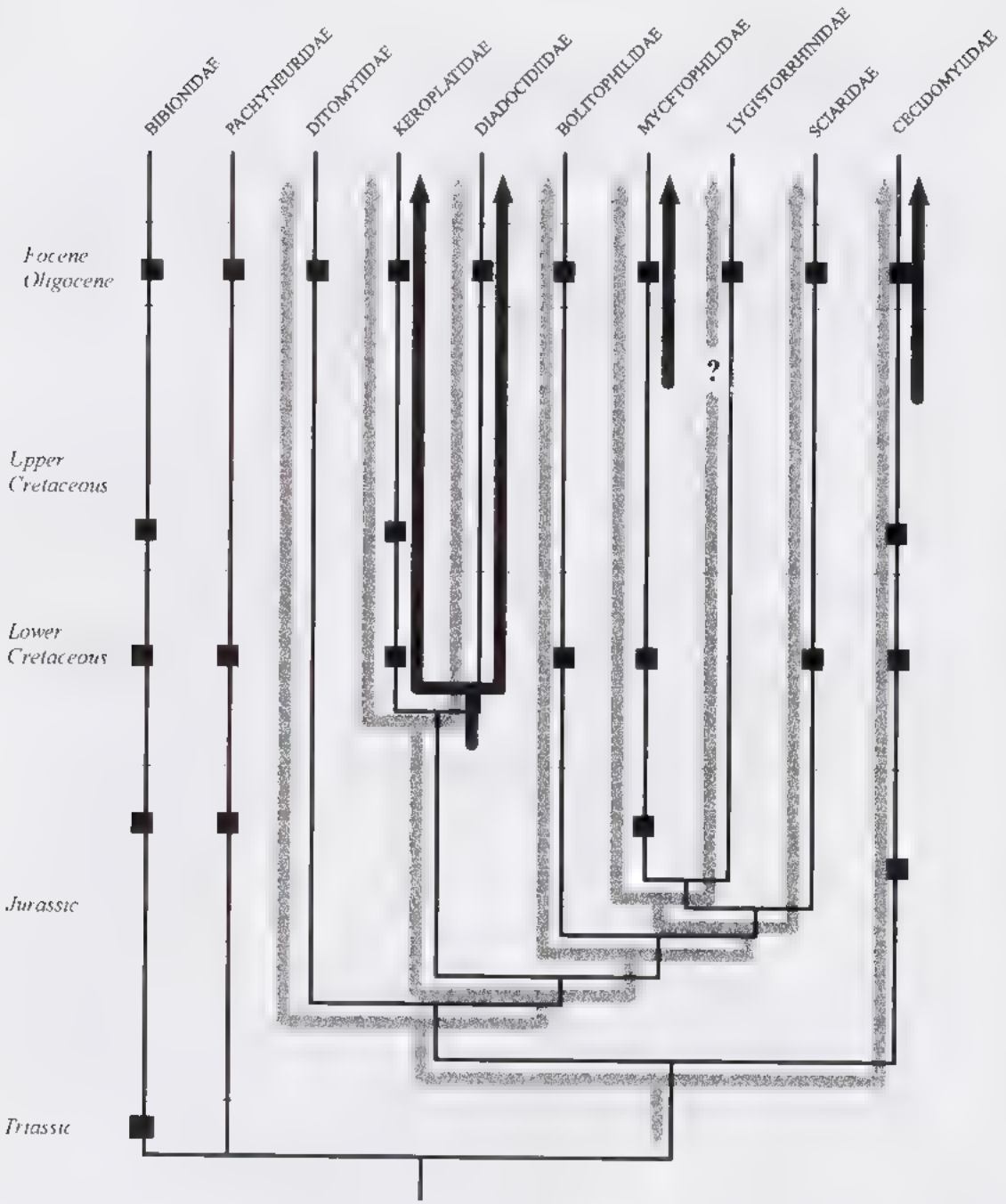


Fig. 7. Temporal evolution of fungivory (light grey lines) and epibiosis (dark grey lines) in the Bibionomorpha. Black squares: pre-Miocene fossil data.

MAMAEV (1968, 1975) for the Cecidomyiidae. The Keroplatidae and Diadocidiidae are epibionts but nevertheless possess this endobiont morphology.

Source: MNHN, Paris

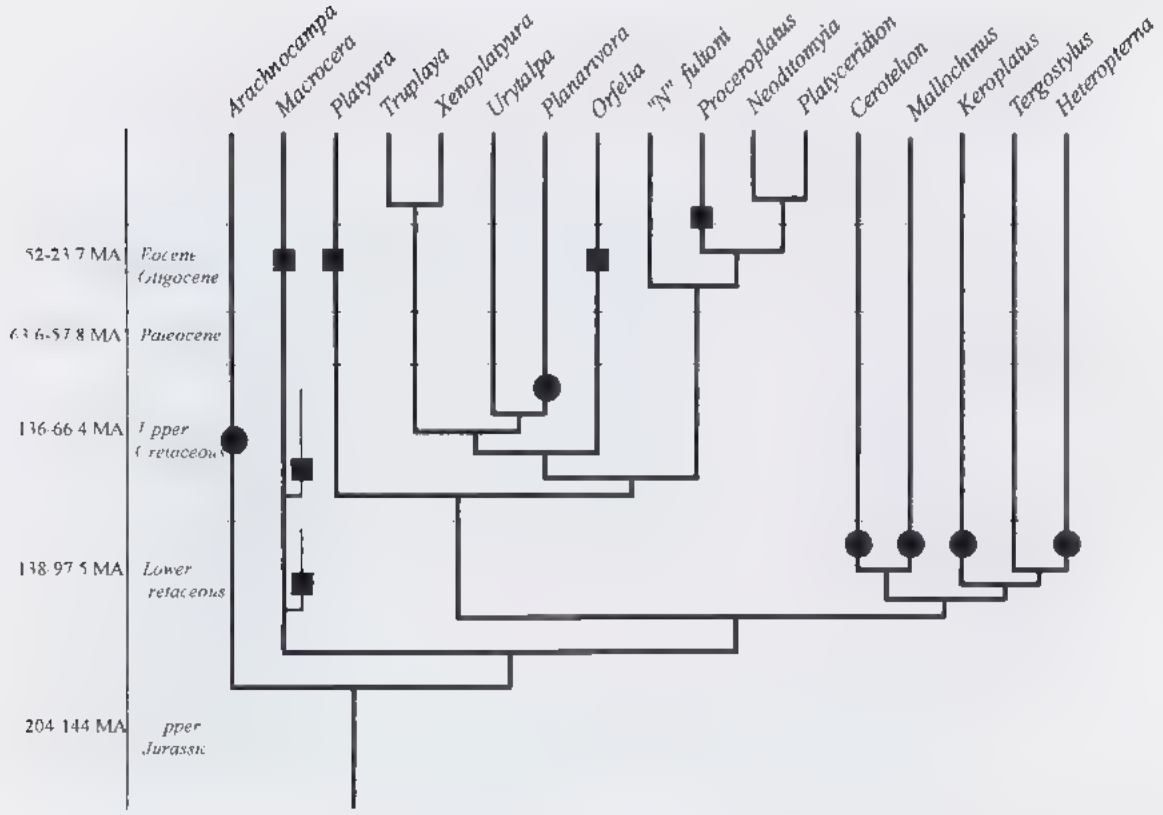


Fig 18 — Pre-Miocene fossil data (black square) and biogeographical data (black disk) available on the taxa studied in this paper *Ara = Arachnocampa; Mac = Macrocera, Pla = Platyura, Tru = Truplaya, Xen = Xenoplatyura; Ury = Urytalpa; Plan = Planarvora, Orf = Orfelia; Neo = Neoplatyura, Pro = Proceroplatus; Neod = Neoditomyia; Plat = Platyceridion, Cer = Cerotelion; Mal = Mallochinus, Ker = Keroplatus, Ter = Tergostylus, Het = Heteropterna* Geologic time scale after EVENHUIS (1994)

Silk secretion is apomorphic for Sciaroidea, it appeared during the Jurassic and was subsequently lost in Ditomyiidae and Bolitophilidae Epibiosis occurred at least three times, once in the clade Keroplatidae-Diadocidiidae, once in Mycetophilidae and once in Cecidomyiidae

Cryptobiosis is apomorphic for the Keroplatidae, and appeared in the Lower Cretaceous, while optobiosis arose independently three times, once in the Keroplatini during the Lower Cretaceous, once in *Arachnocampa* at least by the Upper Cretaceous, and once again in *Neoditomyia*, probably at a much later time, during the Miocene

Predation arose once in the Keroplatidae at some time during the Lower Cretaceous, and at least twice, at an undetermined period, in Mycetophilidae and Cecidomyiidae The net-like web of the predator forms is not derived from the sheet-like web, as assumed by PLATNICK (1979c), but the opposite

Source MNHN Paris

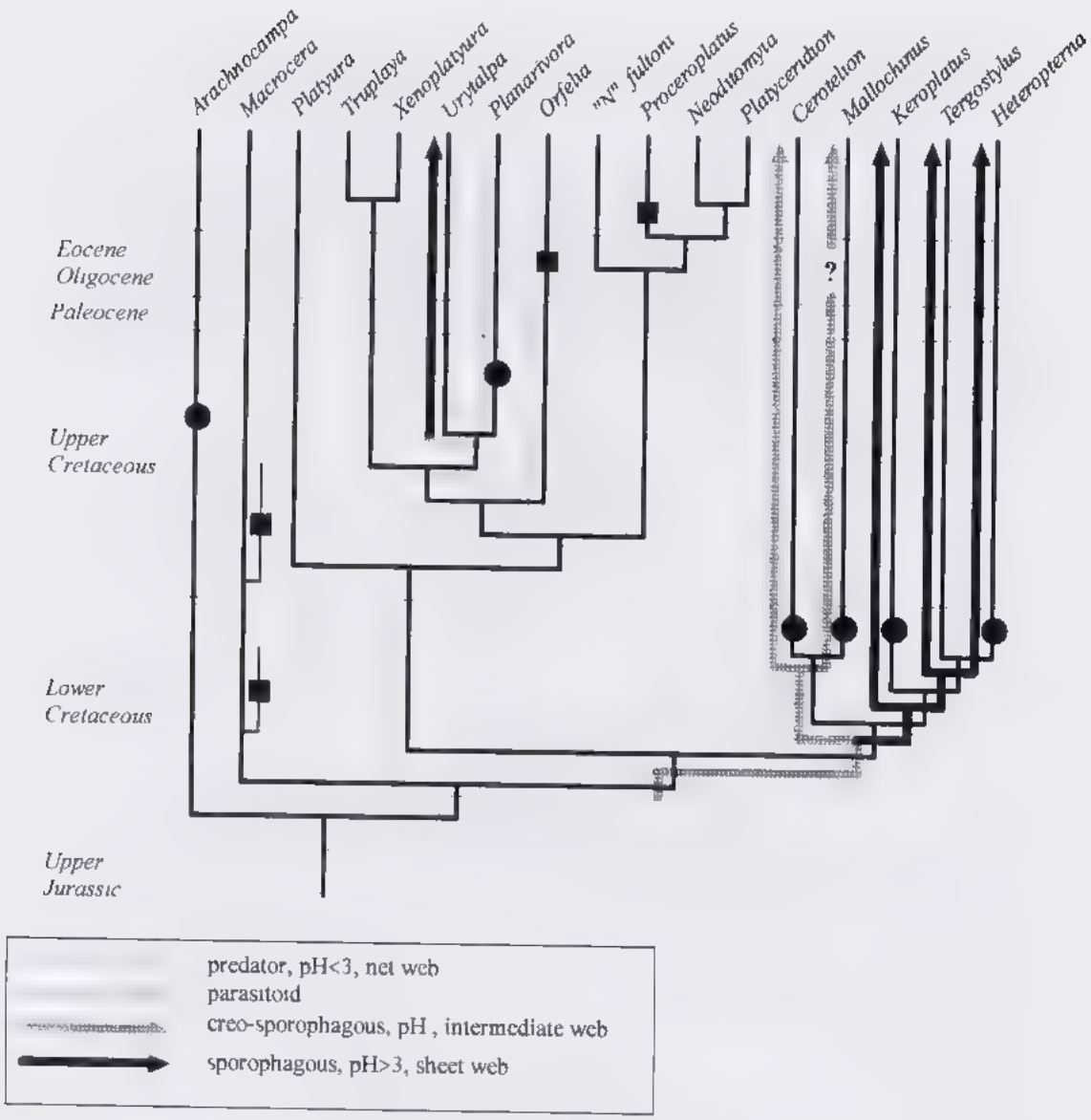


Fig. 19. — Temporal evolution of food preference in the Keroplatidae. See fig. 18 for abbreviations.

Sporophagy in Keroplatidae arose twice, once in the Lower Cretaceous (clade Keroplatini), and once probably in the Upper Cretaceous (genus *Urytalpa*). In these groups, sporophagy seems correlated with a sheet-like web and a labial fluid with a pH of 3 or more. The sporophagy of these Keroplatidae is derived from a predatory diet, and not the opposite as assumed by LASTOVKA (1972). It is not therefore homologous to the "ordinary" fungivory found in other Sciaroidea.

Source: MNHN, Paris

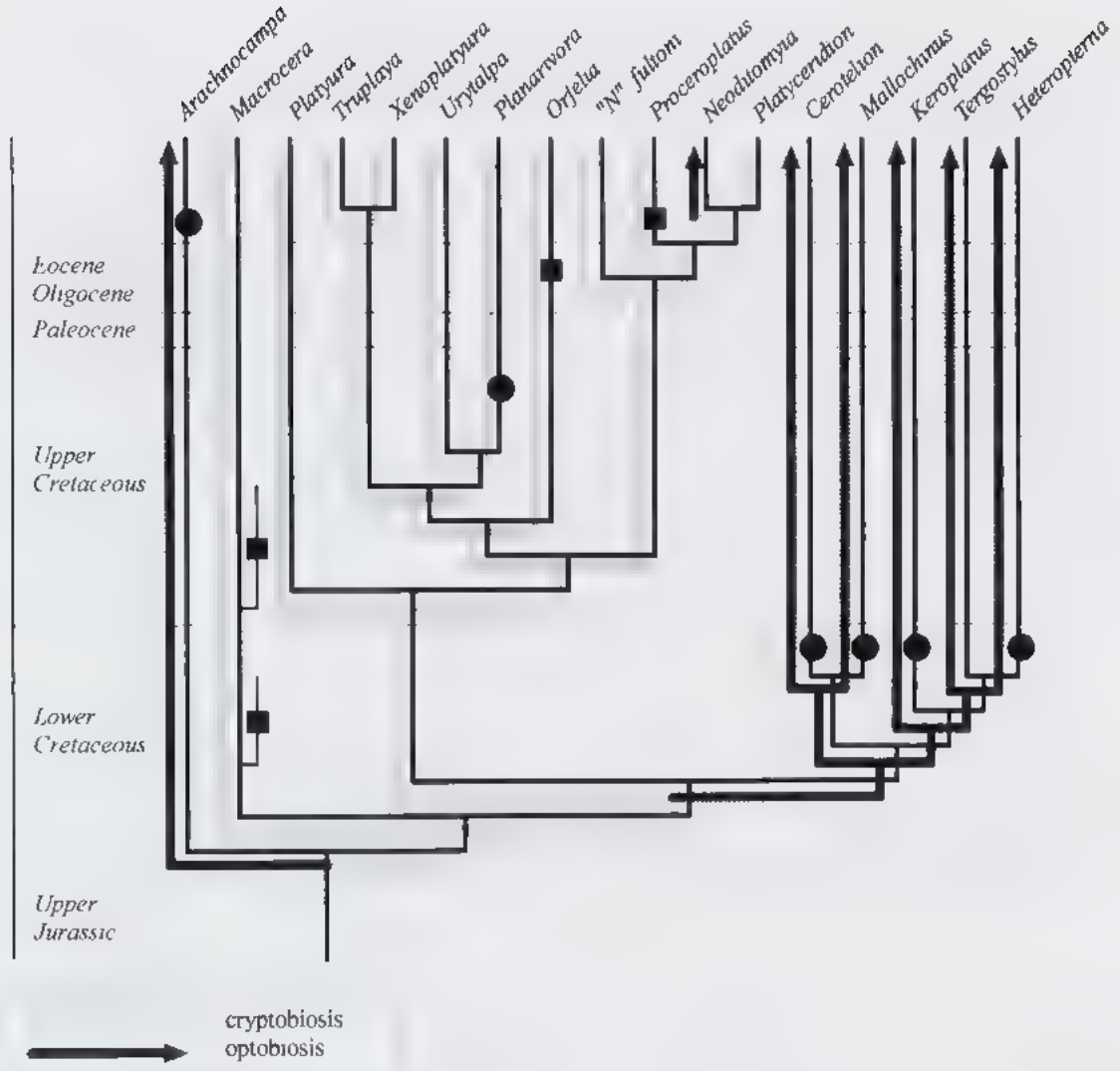


Fig 20 - Temporal evolution of cryptobiosis and optobiosis in the Keroplatidae See fig 18 for abbreviations

We may consider the ancestral larval stock of the Sciaroidea as an endobiont fungicolous insect, without silk secretion, perhaps living as far back as the Upper Jurassic - fossil Sciaroidea have been described as early as the Lower Jurassic (see KALUGINA & KOVALEV, 1985, KOVALEV, 1987b), and of these at least the Pleciofungivoridae certainly are correctly placed in the superfamily The keroplatidian clade, the larvae of which have an endobiont morphology (antennae, eyes and other sensorial organs reduced, scraper mouthparts), became epibionts during the Lower Cretaceous and were then able to exploit at the best their silk- and oxalic-secreting capacity and adopt a predatory diet - making up for the lack of predatory organs by an extension of the body the hunting net-web The extension of the net-web to a sheet-web, an

Source MNHN Paris

intermediary state of which still can observed in *Cerotelion*, and a variant in *Urytalpa*, allowed them to switch to a new food source the collecting of bracket fungi spores

The most important problem posed by the hypothesis of Cretaceous sporophagy in Keroplatini is that the oldest and certain fossil Polyporaceae are only known from the Miocene Some "bracket-fungi" have been described from earlier periods, back to the Carboniferous, but that they really belong to Polyporaceae is apparently still disputed Nonetheless, the genus *Fomites*, described from the Lower Miocene of Lybia, is closely allied with the present genus *Fomes* (LOCQUIN & KOENIGER, 1981), and the ancestral stock of the Polyporaceae should therefore be much older If the present hypothesis is founded, this family of fungi should have appeared at least by the Lower Cretaceous

Although we know many fossils of Bibionomorpha from the Lower and Middle Jurassic, none is close to the Keroplatidae One may therefore think that the acquisition of all these attributes occurred during a very short period of time, no more than a few tens of MY, between the end of the Jurassic and the beginning of the Lower Cretaceous It was followed by a stasis more than 100 MY long, marked only by the divergence of the clade *Urytalpa-Planarivora* towards sporophagy for the first, parasitism for the second, probably during the Upper Cretaceous

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Source: MNHN, Paris

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APPENDIX 1 — List of the characters used in the study of the Sciaroidea (plesiomorphic state 0, apomorphic states 1, 2) (WB = WOOD & BORKENT, 1989, LM = MATILE, 1990, OC = OOSTERBROEK & COURTNEY, 1995) and character matrix.

Larva (unknown for Lygistorrhinidae)

1 Antennae with several segments 0. Antennae one-segmented, cylindrical or disc-like 1 (LM: 386, char. 9 for Bolitophilidae; OC: 295, char. 15).

2 Frontoclypeal apotome short, not extended to posterior margin 0. Frontoclypeal apotome long, extending to posterior margin of head capsule: 1 (LM: 385, char. 2).

3 Posterior tentorium as a rod independent from the head capsule from the metatentorina on 0. Posterior tentorium laterally fused with the head capsule, forming a transverse bridge, unsclerotinized at least at middle 1. The "posterior bridge" of the Sciaroidea is here interpreted as homologous to the posterior tentorium, as demonstrated by its muscles insertion and the discovery of the metatentorina (MATILE, 1967, 1990, see also discussion in OC: 308, char. 101). The condition is variable in Cecidomyiidae (*cf.* MAMAEV & KRIVOSHEINA, 1965, 1993).

4 Anterior tentorial arms strong 0. Anterior arms thread-like 1 (see discussion in OC: 308, char. 02). The condition is variable in Cecidomyiidae.

5 Maxillae pyramidal 0. Maxillae flattened and strongly sclerotinized 1. TESKEY (1981) has pointed out that the maxillae of Nematocera are mostly membranous and passive, and that "a notable exception" was found in the larvae of Sciaroidea. In fact, maxillae of Ditomyiidae resemble closely those of Bibionidae (see also discussion in WB: 1353).

6 Maxillae cardo free 0. Cardo fused or closely apressed to anterior margin of head capsule 1 (WB: 1351, char. 27, OC: 296, char. 25).

7 Maxillary palpus jutting out 0. Palpus reduced, flush with the maxilla 1 (WB: 1356, char. 45, in analysis of Psychodomorpha, LM: 385, char. 3, OC: 296, char. 26).

8 Mandible of the ordinary, pyramidal sort 0. Mandible as a half-circle, toothed at margin, one or more rows of spinules 1. Character not formally included in LM analysis, but dicussed p. 386.

9 Body cylindrical, more or less flattened 0. Body strongly constricted 1. In Bibionomorpha the state 1 of this character exists only in Pachyneuridae and Ditomyiidae, and I think it is an adaptation to life in wood or ligneous bracket-fungi, therefore apomorphic.

10 Subanal region without appendices or macrochaetae 0. Subanal region with one or the other 1. Same than character 9.

11 Metathoracic spiracle present 0. Metathoracic spiracle absent: 1 (WB: 1351, char. 30).

12 Abdominal spiracle VIII present 0. Spiracle VIII absent 1 (STEFFAN, 1981, LM: 385, char. 4).

13 Other abdominal spiracles present and open 0. Abdominal spiracle absent or closed (1) (LM: 385, char. 6).

14 Tracheal system at least 5 dorsal transverse connectives 0. At most one connective 1 (OC: 302, char. 53). State of character unknown in Pachyneuridae and Bibionidae.

Pupa (unknown for Lygistorrhinidae)

15 Prothoracic horns large 0. Prothoracic horns small 1. Character not formally included in LM analysis, but discussed p. 385. Thoracic horns are well developed in Bibionidae, Ditomyiidae and Cecidomyiidae as well as in most other Nematocera (see f. ex. BRAUNS, 1954b).

Imago

16 Ocular bridge absent 0. Ocular bridge present 1 (WB: 1352, char. 33). The condition is variable in Ditomyiidae and Mycetophilidae, where a few genera have an ocular bridge, see also discussion in OC: 309, char. 104. Some Sciaridae and Cecidomyiidae have no eye-bridge, but this is obviously a loss, and the character has been coded 1 in these two families.

Source: MNHN, Paris

17. No tibial spurs II-III: 0. Tibial spurs present: 1. Spurs II-III are absent in some genera of Mycetophilidae, Keroplatidae and Sciaridae, this is again obviously a loss, and the charcater has been coded 1 in these three families. See discussion in LM: 418, A.3.3.
18. Vein R4 long: 0. R4 short or absent: 1 (LM: 385, char: 1, also: 434, A.4.5.8.)
19. Two basal cells: 0. One basal cell: 1. This polarisation is obvious from the dipterous wing groundplan, see for example McAlpine, 1981: 31 *& ff*.
20. Transverse *tb* subvertical: 0. *tb* longitudinal: 1 (LM: 386, char: 5, also: 426, A.4.5.)
21. Transverse *ta* subvertical: 0. *ta* longitudinal: 1 (LM: 386, char: 8, also: 426, A.4.5.). The condition of this transverse, when recognizable, is variable in Cecidomyiidae.
22. Transverse *mcu* present: 0. *mcu* absent: 1 (LM: 386, char: 7).
23. Costa continuous around wing: 0. Costa abbreviated at apex of wing: 1 (OC: 304).
24. Insertion of abdomen on thorax wide: 0. Insertion narrow: 1. Insertion very narrow: 2 (LM: 386, char: 12, also p. 379-383).
25. Mediotergite partly included in abdominal segment I: 0. Mediotergite free: 1. Character not formally included in LM analysis, but dicussed p. 379-383.
26. Laterotergite narrow: 0. Laterotergite wide: 1 (LM: 386, char: 11).
27. Coxae short: 0. Coxae long: 1. See discusssion in LM: 413, A.3.1.
28. Sclerotized part of aedeagus tubular: 0. Aedeagus flattened: 1 (Wood, 1991, OC: 307, char: 92).
29. Somatic and germ cells with same number of chromosoms: 0. Some chromosomes eliminated in somatic cells: 1 (WB: 1352, char: 32, OC: 307, char: 98). State of character known only in some species of Sciaridae and Cecidomyiidae, and one species of Mycetophilidae.
30. Central microtubule of sperm tail present: 0. Central microtubule absent: 1 (OC: 307: char: 95 – polarity inversed in text but not in character matrix). Keroplatidae polymorphic (coded ?). State of character unknown in Bibionidae, Pachyneuridae, Ditomyiidae and Lygistorrhinidae.

	123456789111111111122222222223 01234567890123456789
Bibionidae	0000000000000?0010000000000??
Pachyneuridae	0000000011100?0010000000000??
Ditomyiidae	0011011011100007101000111011??
Diadocidiidae	1111111000111010111000111111??
Keroplatidae	1111111000111010111000121111??
Bolitophilidae	1111111100110010111100111101??
Mycetophilidae	1111111100110017111111121111??
Lygistorrhinidae	??????????????701111111211111??
Sciaridae	1111111000110111111111110010111
Cecidomyiidae	00??0100001001010111?100000111

Appendix 2 – List of the characters used in the study of the subfamilies of Keroplatidae and tribes of Keroplatinae (plesiomorphic state: 0; apomorphic state: 1, LM = Matile, 1990) and character matrix.

Larva

1. Head capsule short and round: 0. Head capsule short and rectangular: 1. Head capsule long and narrowed in middle: 2 (LM: 473).
2. Anterior tentorial arms very thick: 0. Anterior tentorial arms reduced: 1. Anterior tentorial arm strongly reduced: 2.
3. One pair of stemmata: 0. Two pairs of stemmata: 1 (LM: 474).
4. Antennae small: 0. Antennae large, reaching almost middle of genae: 1 (LM: 475).
5. No lateral labral lobes: 0. Two labral lateral lobes: 1 (LM: 475).

Source: MNHN, Paris

6. Maxillary cardo normal. 0. Maxillary cardo strongly lenghtened. 1 (LM 475)
7. Mandibles short, Bibionid-like. 0. Mandibles long and narrow. 1 (LM 371)
8. Larva hemipneustic. 0. Spiracles absent or non fonctional. 1 (LM 371)
9. Abdominal spiracles present, either opened or closed. 0. No abdominal spiracles. 1 (LM 477)
10. Malpighian tubules normal. 0. Malpigian system cryptonephridian. 1 (LM. 371).
11. Oesophagus short. 0. Oesophagus as long as middle gut. 1 (LM. 371)
12. SII sensilla present. 0. SII sensilla absent. 1 (LM. 371).
13. Abdomen smooth, without hypodermal colored bands. 0. Abdomen finely annelated, with colored hypodermal bands. 1 (LM. 371).
14. No luminous organ, or a simple luminous organ linked to fat body or black bodies. 0. A complex luminous organ formed by the Malpighian tubules and a tracheal reflector. 1 (LM. 477)

Pupa

15. Nototheca simple. 0. Nototheca with a sagittal crest. 1 (LM. 473)

Imago

16. Foramen magnum in dorsal position. 0. Foramen magnum in central position. 1 (LM, 1990. 387)
17. Mediocellar sclerite absent. 0. mediocellar sclerite present. 1 (LM. 389)
18. No cerebral sclerite. 0. A cerebral sclerite. 1 (LM. 388)
19. Antennae simple. 0. Antennae thickened or pectinated. 1 (LM. 395). The state of character is variable in Orfelina.
20. Postmentum present. 0. Postmentum absent. 1 (LM. 398). The condition is variable in Orfelina.
21. Four palpomeres, if less than 4 the last one not thickened and porrect. 0. One or two palpomeres, the last one thickened and porrect. 1 (LM. 397).
22. Presternite present. 0. Presternite absent. 1 (LM. 401)
23. Transverse suture complete. 0. Transverse suture incomplete. 1 (LM. 402).
24. Postpronotum lateral and distinct. 0. Postpronotum dorsal, more or less fused with praescutum. 1 (LM. 399).
25. Mesepimeron almost as wide ventrally than dorsally. 0. Mesepimeron narrow or absent ventrally. 1 (LM. 411)
26. Metepimeron almost as wide as high. 0. Metepimeron much wider than high. 1 (LM, 1990. 412, the epimeron and episternite have been inadvertently inversed while lettering fig. 1080-1087).
27. Laterotergite narrow and subvertical. 0. Laterotergite wide and and oblique. 1 (LM, 1990. 406)
28. Alular incision present. 0. Alular incision absent. . (LM, 1990. 426, the presence of an alular incison is here considered as the groundplan of Sciaroidea, its disappearance in *Arachnocampa* and *Bolitophila* a reversal)
29. Costal vein extending after apex of wing. 0. Costa shorter. 1 (LM, 1990. 431).
30. R4 present or absent, R1 and R5 close to one another. 0. R4 absent, and at the same time R1-R5 widely separated. (LM. 435).
31. No radiomedian coaptation. 0. A radiomedian coaptation. 1 (LM. 436). State variable in Ditomyiidae.
32. Basal and *ta* transverse fused. 0. Basal transverse distinct from *ta*. 1 (LM. 438).
33. Coxae of about the same length. 0. Coxae I longer than the two other pairs. 1 (LM. 414).
34. Tibiae bearing macrochaetae. 0. No tibial setae. 1 (LM. 417)
35. Tibiae II-III with apical combs. 0. No apical tibial combs. 1 (LM. 421).
36. A pair of spiracle on abdominal segment I. 0. No abdominal spiracles I (LM. 448)
37. male. segment VIII about half as long as VII. 0. Segment VIII shorter, more or less retracted under VII. 1 (LM. 450).
38. male. Hypoproct complete or membranous, but not notched basally. 0. Hypoproct deeply notched basally. 1 (LM. 452)
39. male. Sternite IX distinct. 0. Sternite IX fused or lost. 1 (LM. 456)
40. male. Tergite X present. 0. Tergite X absent. 1 (LM. 451). Condition ambigous in Ditomyiidae (LM. 452)
41. male. Ejaculatory apodeme developed. 0. Ejaculatory apodeme reduced to a dorsal rod. 1 (LM. 469)
42. female. Tergite VIII entire or weakly reduced. 0. Tergite VIII strongly reduced or membranous, invaginated under VII. 1 (LM. 471)
43. female. Sternite VIII complete basally. 0. Sternite VIII completely separated in two halves. 1 (LM. 471).
44. female. Tergite IX entire. 0. Tergite IX reduced. 1. Tergite IX entirely membranous. 2 (LM. 471).
45. female. Cerci two-segmented. 0. Cerci one-segmented. 1 (LM. 472).

Source: MNHN, Paris

	12345678911111111112222222222333333333344444 01234567890123456789012345678901234
Ditomyiidae	00000000000000000000001111000070000110070000
Arachnocampinae	20100011111111111001010000010101000000101100
Marocerinae	11011110111100001000011101010101110110100010
Keroplatini	11001011011100000101011111010100001110100111
Orfeliini	11001011011100000??001111101010000111010010

APPENDIX 3 List of the characters of 5 genera of Keroplatini (plesiomorphic state 0, apomorphic states 1 2, LM = MATILE, 1990) and character matrix

Imago

1 Eyes not or slightly emarginated above antennae: 0 Eyes strongly emarginated. 1 (LM 390).
2 Antennal scape beakless. 0. Antennal scape with a beak. 1 (LM 394)
3 Four palpomeres 0 Two palpomeres: 1 One palpomere 2 (LM 396)
4 Mouthparts long jutting out from the lower eye margin 0 Mouthparts short, not jutting out from eye margin 1 (LM 398)
5 Scutellum haired on entire disk 0 A pair of discal setae 1 Scutellum bare on disk 2 (LM 404)
6 Laterotergite haired 0. Laterotergite bare. 1 (LM 407).
7 Tibial setuale irregular 0 Tibial setae in regular rows 1 (LM. 416)
8 Abdomen with an intercalar sclerite. 0 No intercalar abdominal sclerite 1 (LM 450)
9. male abdomen cylindrical 0 Abdomen flattened. 1 (LM 445)
10 male tergite IX as a flat plate 0 Tergite IX expanded laterally 1 (LM 445)
11 male: hypoproct at least partially sclerotinized. 0 Hypoproct entirely membranous: 1 (LM 452)
12 male perigonostylar bridge complete 0 Perigonostylar bridge incomplete: 1 (LM 458)
13 male inner margin of gonostyle not more sclerotinized than rest of appendice 0 Inner margin of gonostyle strongly sclerotinized and denticulated. 1 (LM 466)
14 female sternite VIII infolded at most on basal half 0 Sternite VIII infolded at least at ¾ of its length 1 (LM 471)

	12345678911111 01234
Arachnocampa	00002000000000
Cerotelion	11101100000010
Mallochinus	11101000000010
Keroplatus	01100011101101
Tergostylus	00210111111101
Heteropterna	00210111110101

APPENDIX 4 List of the characters used in the study of 10 genera of Orfeliini (plesiomorphic state 0, apomorphic states 2, (LM= MATILE, 1990) and character matrix

Imago

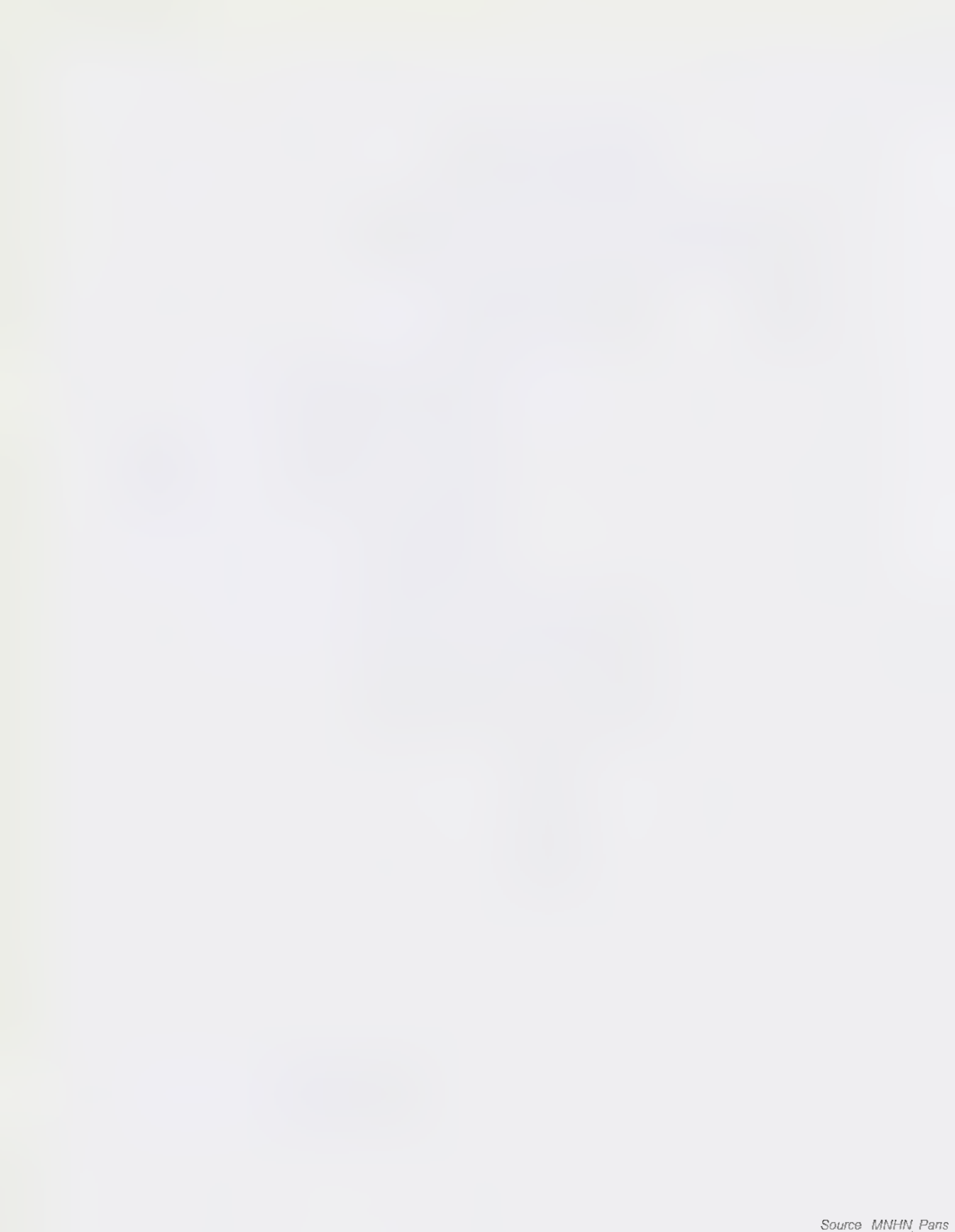
1 Antennae threadlike 0 Antennae pectinated ou serrulated. 1 (LM 395).
2 4 palpomeres. 0 Three palpomeres or less (LM 396)
3 Three ocelli 0 Two ocelli (LM 389)
4 No parachrostical stripes 0. Two wide parachrostical stripes 1 (LM 403).
5 Prospiracular setae present 0 Prospiracular setae absent. 1 (LM. 410)
6 Mediotergite rounded at apex 0 Mediotergite strongly angulous. 1 (LM 405)
7 Laterotergite setiferous 0 Laterotergite bare 1 (LM. 407).

Source MNHN Paris

8 Mesanepisternite setiferous 0 Mesanepisternite bare (LM 410)
9 Metanepisternite setiferous 0 Metanepisernite bare 1 (LM 412)
10 C continuing widely after tip of R5 0 C stopping at tip of R5, or a little farther 1 (LM 431)
11. M2 and M3 entire 0 M2 et M3 interrupted before wing margin 1 (LM 438)
12 Basis of M present as a true vein 0. Basis of M absent or present as a fold 1 (LM 438)
13 Radiomedian fusion bearing setulae 0 Radiomedian fusion bare 1 (LM 438).
14 Lower veins haired dorsally 0 Lower veins bare 1 (LM 442)
15 Anal vein long 0 Anal vein short 1 (LM 441).
16 Tibial setulae irregular 0 In regular rows 1 Some darker rows of closely set setulae 2 (LM 416)
17 Outer tibial spurs II-III long 0 Outer tibial spurs II-III reduced (LM 418)
18 male abdomen cylindrical 0 Abdomen petiolated and laterally compressed 1 (LM 445).
19 male hypopygium capable of rotation 0 hypopygium non rotatable 1 (LM 67, 451).
20 male Tergite IX long or short, but as a dorsal plate 0 Tergite IX long or very long, strongly expanded laterally 1 (LM 456)
21. male aedeagus short 0. Aedeagus long, extending at least to tergite VII (LM 469)
22 male gonostyles with one segment, either divided or not 0 Gonostyles at least two-segmented 1 (LM 465)
23 male gonocoxites approximated ventrally 0 Gonocoxites widely separated ventrally, the aperture closed by a ventral desclerotized process 1 (LM 461)

	12345678911111111112222 01234567890123
Arachnocampa	00011001110001000000000
Platyura	00011011110010000000000
Orfelia	00001111100101120000010
Neoditomyia	10100000100101111000000
Xenoplatyura	00001111001101000111111
Urytalpa	00011011110100000111100
Truplaya	00000111111101010111111
Proceroplatus	10001000100111011000000
Platyceridion	11101000102111011000010
Planarivora	01010110100110000011110
"N." fultoni	10001010100111010010000

Source: MNHN, Paris



Source: MNHN, Paris

The Probabilistic Inference of Unknown Data in Phylogenetic Analysis

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ABSTRACT

Unknown characters and attributes are inferred in phylogenies using a probabilistic method. The probability of the position of fossil taxa having uncertain relationships because of the lack of unambiguous synapomorphies can be calculated using a similar method. These methods allow a better definition of the limits of actualism in Paleontology and can be applied to palaeoclimatic and palaeoenvironment studies.

RESUME

Inférence probabiliste de données inconnues en analyse phylogénétique

Il est propose une methode probabiliste d'inférence phylogenétique de caracteres et attributs d'etat inconnu ainsi qu'une methode analogue de calcul de la probabilite de la position de taxa fossiles *a priori* incertaine par manque de synapomorphies non ambiguës. Ces methodes permettent de quantifier les hypotheses d'inference basees sur les liens de parenté des taxa fossiles et ainsi de definir les limites de l'utilisation du principe de l'actualisme en paleontologie en particulier pour la paleoclimatologie et les analyses paleoenvironnementales.

INTRODUCTION

One of the main problems in Paleontology is the reconstruction of the palaeobiotas and palaeoclimates by comparison between fossil and recent taxa. One can use the actualist method which extends the Recent biological and ecological data to the past. FURON (1964) gave an example which represents a good summary of the use of actualism: "Les grands Foraminiferes [ ] vivaient dans des mers chaudes [ ] mais c'est évidemment la repartition des récifs coralliens qui nous donne les renseignements les plus précis". But incorrect use of actualism could be misleading and several examples reveal particularly unreliable for the study of very old palaeoenvironments. After BRYANT & RUSSELL (1992), there are two different methods of inference of Recent data to the past: a) after the morpho-functional inference theory, a fossil organ identical to a Recent one had a similar function ; b) after the phylogenetic inference theory,

NEL, A., 1997. The probabilistic inference of unknown data in phylogenetic analysis. *In* GRANDCOLAS, P. (ed.), The Origin of Biodiversity in Insects: Phylogenetic Tests of Evolutionary Scenarios. *Mem. Mus. natn. Hist. nat.*, **173**: 305-327. Paris. ISBN: 2-85653-508-9

Source: MNHN, Paris

a fossil taxon related to a Recent one had a similar biota, under a similar climate and environment. Being limited to functional data, the first type of inference is often useless for palaeoclimatic analysis. Furthermore, confusions between the two types of inference are frequent and are leading to abusive conclusions, supported by weak evidences. Because of the lack of clear synapomorphies with Recent groups, the phylogenetic positions of fossil taxa are frequently very uncertain. Evenly, one would be tempted to use phenetic methods for classifying these taxa. Either BRYANT & RUSSELL's method (*loc. cit.*) only give qualitative phylogenetic inferences. As methods of quantification of data inferences and of phylogenetic positions of taxa are lacking, we have attempted to define them with a probabilistic theory based on cladistic analysis. We did not attempted to use a maximum likelihood method of analysis because the informations concerning the various probabilities for the ancestor state, change of states along the branches, etc. (DARLU & TASSY, 1993), are never available for the inference of complex palaeoclimates or palaeoenvironmental informations.

DATA INFERENCES (FOSSIL / RECENT)

Using phylogenetic systematics, it is possible to extend informations from Recent data to the fossil record on the basis of the systematic position of the fossil taxon. Informations (of palaeoclimatic and palaeoenvironmental types between others) are then to be considered as attributes (*sensu* MICKEVICH & WELLER, 1990, DELEPORTE, 1993, GRANDCOLAS, 1993, GRANDCOLAS *et al.*, 1994). An attribute is a trait of extrinsic type. Its primary homology (*sensu* DE PINNA, 1991, before any phylogenetic analysis) is not assessed, but its similarity can be postulated, in order to give the same name to traits of the different taxa. A character is, in phylogenetic analysis, a trait unambiguously homologous in several taxa before any the phylogenetic analysis. The polarization of an attribute is to be made by optimization on the phylogenetic tree. BRYANT & RUSSELL (1992) have established a general method of inference of characters (or attributes), the states of which are unknown for taxa already included in a phylogenetic analysis: if the taxon (fossil or not) is included in a group having an homogeneous state for the considered attribute (or character), the probability for the (fossil) taxon to have an information homologous to the data given by the Recent taxa increases. On the contrary, if the (fossil) taxon is only the sister-group of a Recent group, the inference of information becomes more uncertain and cannot be justified with the sole hypothesis of parsimony. Only an hypothesis of phylogenetic proximity provides support for the hypothesis of inference. This method of inference only tests for presence/absence of a character in one taxon while it is present in other taxa. It does not concern the possible autapomorphies of the fossil taxon for the studied attribute. It is impossible to infer phylogenetically an unknown autapomorphic character for a fossil taxon, on the basis of characters of different type of the nearest Recent relatives. "Phylogenetic inference is conservative" (BRYANT & RUSSELL, 1992).

The method of BRYANT & RUSSELL is based on the outgroup "ascendant" algorithm of MADDISON *et al.* (1984): the situation of a character (or an attribute) at each internal node of the tree is parsimoniously inferred by the situations at the two immediately adjacent nodes. For a character X with two states "a" and "b", an internal node is labeled "a" if the two immediate adjacent nodes are labeled "a" and "a" or "a" and "a or b". Symmetrical situation occurs with "b". Nodes are labeled "a or b" if the adjacent nodes are labeled "a" and "b", or "a or b" and "a or b". The external node with the missing information is then supposed to share the same state as

Source: MNHN, Paris

the immediately internal adjacent node This method is problematic for the inference of characters that have been used in the construction of the minimal tree, using adequate softwares because the computer programs Hennig86 and Paup 3 1 1 trend to affect definite values to the missing entries, even if they do this in very different ways in Paup, "only those characters that have non-missing values" are supposed to "affect the location of any taxon on the tree" (SWOFFORD, 1990) because Paup assigns to the taxon affected by the missing character the character state that would be most parsimonious given its placement in the tree Equally parsimonious trees are constructed for the concerned character and then discriminated on the other non missing characters PLATNICK *et al* (1991) have tested Hennig86 and Paup and would confirm the assumption of SWOFFORD They add that Hennig86 trends to attribute global peculiar states to missing data, depending of the tree topology Paup gives a less resolved solution than Hennig86, because it interpret the missing data in the construction of the minimal trees Nevertheless, if the missing data are reinterpreted and used in the construction of the minimal tree, it is delicate to test their value on the basis of the same tree This difficulty does not exist if the concerned character is considered as an attribute, not included in the preliminary construction of the tree and independently tested after this construction

BRYANT & RUSSELL could not quantify their hypothesis of congruence, but it is possible to calculate the probability of the following event. [the missing information is homologous to the information given by the nearest relative taxa] A preliminary hypothesis is necessary the inference of the unknown situations for the F-taxa will not add homoplasies or steps to the general shape of the concerned attribute in the minimal tree. All the situations that do not imply supplementary homoplasies or supplementary steps are then supposed to be equally probable Following this condition, the probability of the event [the studied taxon has a peculiar state for the studied character] can be calculated by making the ratio of the number of favorable situations by the total number of possible situations

Bipolar attribute X

Theoretical procedure. X is supposed to have two states "a" and "b" As the polarity of the attribute and its homoplasy rate are completely unknown, we consider that it can equally be in the states "a" or "b" in the root of the tree Then, the minimal scenarios (with the lowest number of steps) that explain the known distribution of the attribute (excluding the F-taxon) are reconstructed for the two situations "state a for the root" (or "root a") and "root b"

Then, two options are possible

option (1) either these two minimal scenarios concerning the possible situations of the root can either be considered as equally probable, even if one can imply more steps than the other

option (2) or the minimal scenarios that explain the known distribution of the attribute with a "root b" is affected of a weight (x), then the minimal scenario for the "root a" has a weight (1 x) They are not considered as equally probable, $0 < (x) \leq 1$ Option (1) is a peculiar case of option (2), with (x) 0 5 Nevertheless, option (1) corresponds to the minimal *a priori* scenario (equal weight for the two situations of the root)

After, the F-taxon is re-included in the tree, then are only accepted the situations where the alleged state for F can be reconstructed without adding supplementary homoplasies or steps in the tree ("favorable cases"), in order to keep the same minimal lengths for the new trees This method of inference do not add other hypothesis than the two possible situations in the root of

Source MNHN, Paris

the tree. Finally, the "favorable cases" are counted, and the probability of the event (F is in the state "a") labeled "p (F . a)" is simply the following ratio

p (F a) (number of cases favorable to the state "a")/(number of favorable cases)

Application 1) If a taxon F is simply the sister-group of a known taxon A, without further information, the probability for the event [F and A share the same state for a character X] = 0,5, which can be written p (F a) 0,5 = p (F b). A simple information of sister-group relationship does not allow any prospective for unknown characters.

2) If a taxon F is the sister-group of a known taxon A1, [F + A1] being the sister-group of a known taxon A2, and if A1 and A2 share the state "a" of the character, unknown for F (Fig. 1).

BRYANT & RUSSEL's method infers the situation "a" for F.

Using the probabilistic method,

with option (1) for the roots, I have

$$p(F : a) = 1 \text{ and } p(F \cdot b) = 0 \quad \text{(Fig. 1)}$$

with option (2) for the roots, I have:

$$p(F : a) = [x + (1 - x)] / [x + (1 - x)] = 1$$

Thus the results of the two methods are congruent, in either situations.

3) If the taxon A2 has the contrary state "b".

After BRYANT & RUSSEL'S method, the inferred situation is "a or b" for F.

Using the new probabilistic method,

with option (1) for the roots, we have:

$$p(F \text{ a}) = 2/3 \text{ and } p(F : b) = 1/3 \text{ (Fig. 2)}$$

with option (2) for the roots, we have, as p (F : a) depends on x:

$$p(F : a)(x) = [x + (1 - x)] / [(x + x + (1 - x)] = 1 / (1 + x)$$

As $0 \leq x \leq 1$, p (F a) (0) 1 , p (F a) (1) 0 5 , and p (F a) (0.5) 2/3 [as x = 0 5 corresponds to option (1)].

More generally, $0.5 \leq p(F \cdot a)(x) \leq 1$

With the minimal scenario for the roots, p (F : a) = 2/3

The results of the two methods are congruent but we can quantify the alternative "a or b".

4) If we add to the schema of Figure 1 the information (Fig. 3) of an out-group (including one or several taxa) A3 which has a state "b" :

BRYANT & RUSSEL's method infers the situation "a" for F.

With the probabilistic method,

with option (1) for the roots, I have

$$p(F : a) = 1 \text{ and } p(F : b) = 0$$

with option (2) for the roots, I have:

$$p(F : a)(x) = [x + (1 - x)] / [x + (1 - x)] = 1$$

The two results are clearly congruent. Adding supplementary taxa which would be branched lower in the phylogenetic tree will not change the probabilities for the terminal branch which includes F.

Source: MNHN, Paris

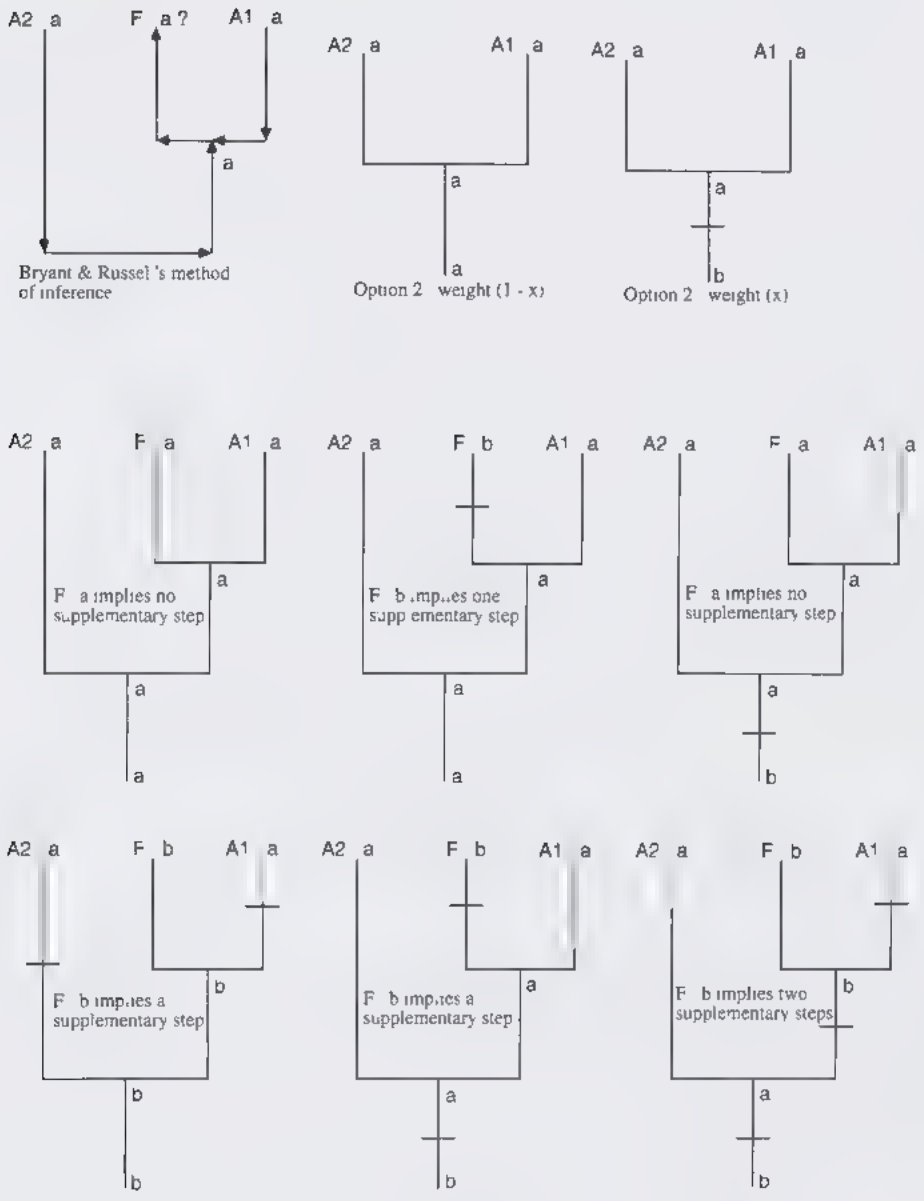

FIG. 1 — p(F a) = 1 ; other situations also imply supplementary steps.

5) But if the ingroup includes different taxa with different states, or polymorphic taxon(a), the probability will decrease. In Figures 4 and 5, we give two different examples.

First example. If a third taxon A3 with the contrary state "b" is added to the situation of Figure 2 (Fig. 4) as sister group of [(A1 + F) + A2], the probability is p (F a) = 1/2 for the two

Source: MNHN, Paris

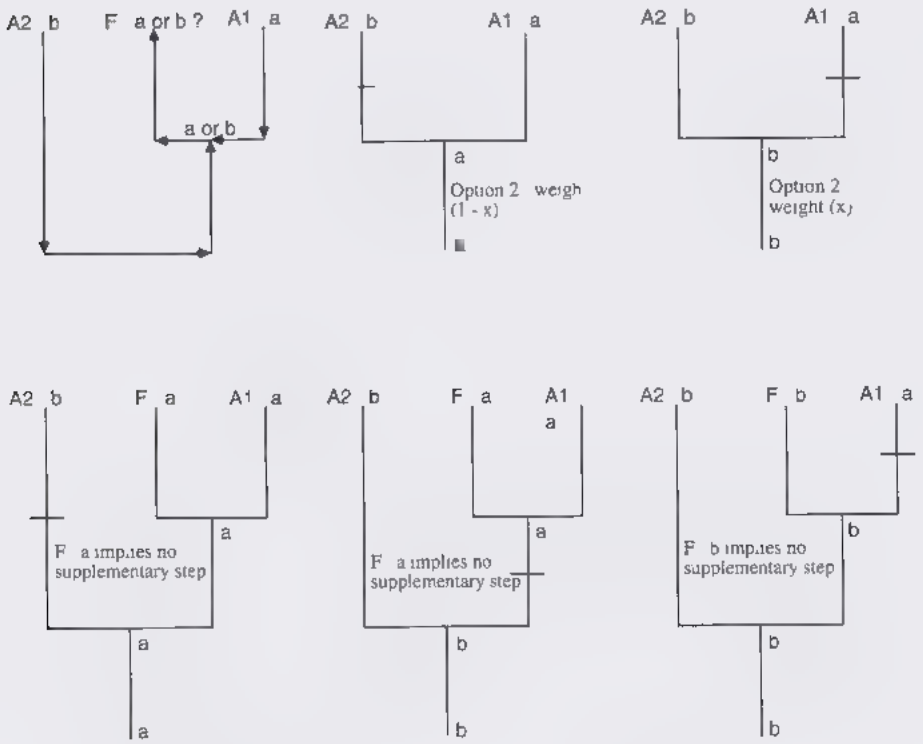

FIG. 2 — p(F a) = 2/3 , other situations imply supplementary steps

options (1) or (2), it has decreased, compared to the result of Figure 2 This result remains congruent with the predictions of BRYANT & RUSSEL's method (inference of "a or b"), but there is a kind of "attraction" of the low branches Nevertheless, if further taxa having the state "b" are added more basally, the result will not change

First example If a third taxon A3 with the contrary state "b" is added (Fig 5) as sister-group of [(A1 + A2) + F]:

BRYANT & RUSSEL's method gives the inference "b" for F.

With probabilistic method, I have

with option (1) for the roots

$$p(F : a) = 1/3$$

with option (2) for the roots:

$$p(F . a)(x) = (1 - x) / [x + (1 - x) + (1 - x)] = (1 - x) / (2 - x)$$

p (F a) (0) = 0 5 , p (F a) (1) = 0 , and p (F a) (0 5) 1/3 (minimal scenario for the roots) More generally $0 \leq p(F\ a)(x) \leq 0\ 5$, because $0 \leq x \leq 1$ and p (F a) is a decreasing function of x

Thus, in this case, the two methods are not congruent, but the probabilistic method agrees with the intuitive assumption of more uncertainty in the inference if the sister taxon (A1 + A2) of F is polymorphic

Source MNHN Paris

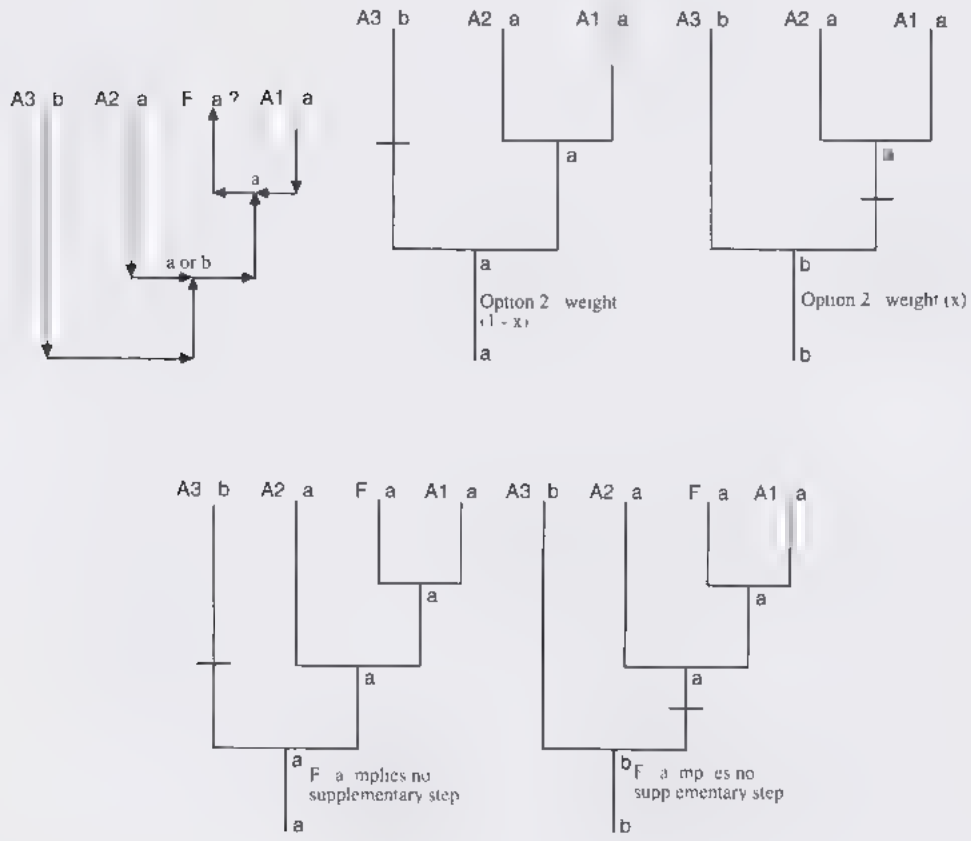

FIG 3 — p(F . a) = 1

6) In the case of several taxa with an unknown state for character X (Figure 6), BRYANT & RUSSELL (*loc. cit.* 410-411, Fig 4) concluded that no peculiar F-taxon is privileged in the inference of the informations My study leads to similar results

In the situation of Figure 6, BRYANT & RUSSEL's method implies the same inference ("state a") for the two taxa F1 and F2

The probabilistic method gives the same probabilities for F1 and F2, for the two options (1) or (2), clearly not depending on the position of the fossil taxa in the tree

$$p(F1 \quad a) = 1 = p(F2 : a)$$

Similar results can be obtained with more F-taxa in similar positions

7) The same calculations have been made in the case of two F-taxa (Fig 7), but with a situation similar to that of Figure 2

Bryant & Russel's method gives the same inference of "a or b" for F1 and F2 but it does not add information to the situation of Figure 2

With probabilistic inference, I have

with option (1) for the roots

$$p(F1 : a) - 3/4 \text{ but } p(F2 . a) = 1/2$$

Source MNHN, Paris

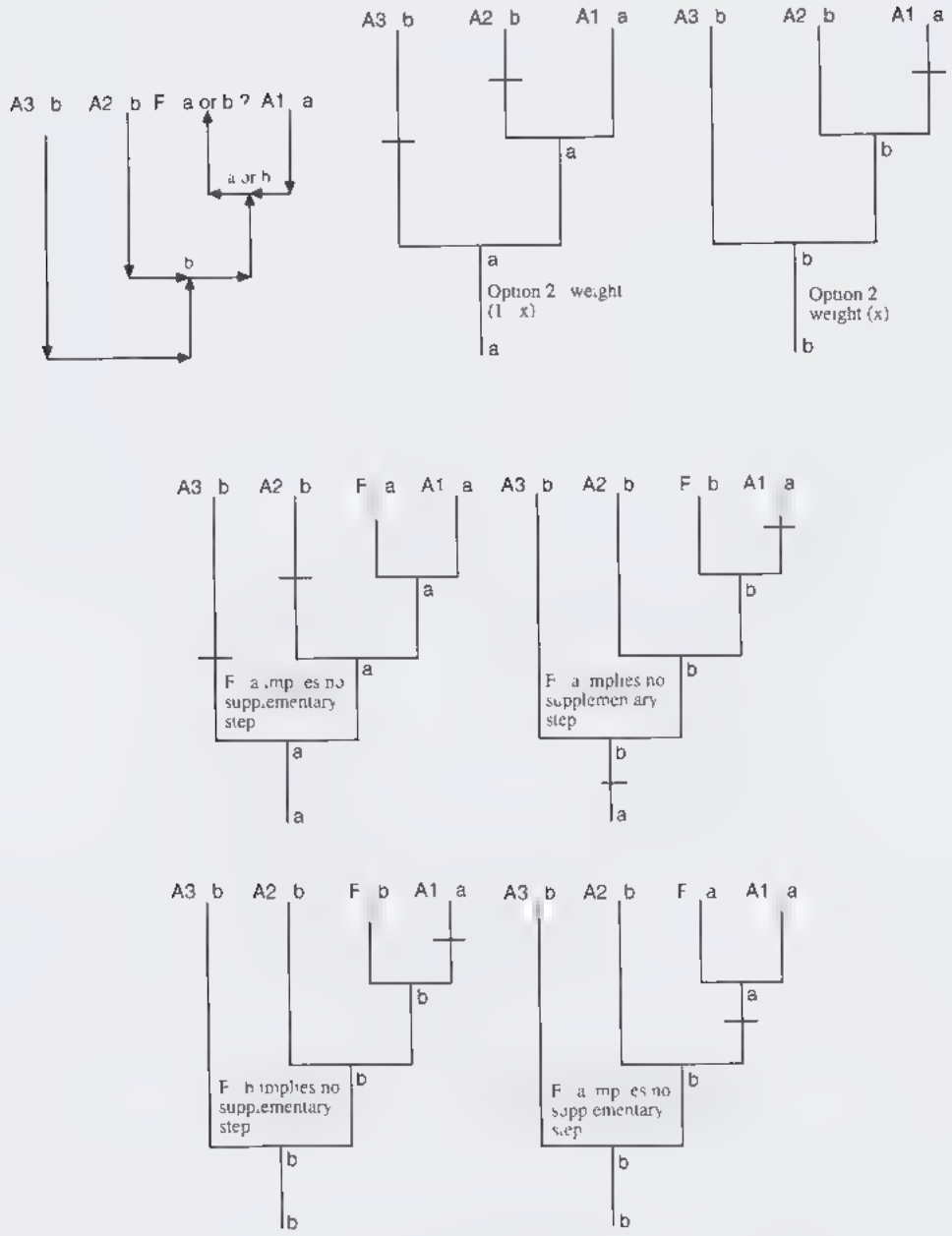

FIG 4 — p(F · a) = 1/2

with option (2) for the roots

p (F1 · a) (x) = (x + 1) / (2x + 1) and p (F2 · a) (x) = 1 / (2x + 1)

p (F1 : a) (0) = 1 ; p (F1 . a) (1) = 2/3 ; and p (F1 · a) (0.5) = 3/4

p (F2 : a) (0) = 1 ; p (F2 · a) (1) = 1/3 , and p (F2 · a) (0 5) = 1/2

Source MNHN, Paris

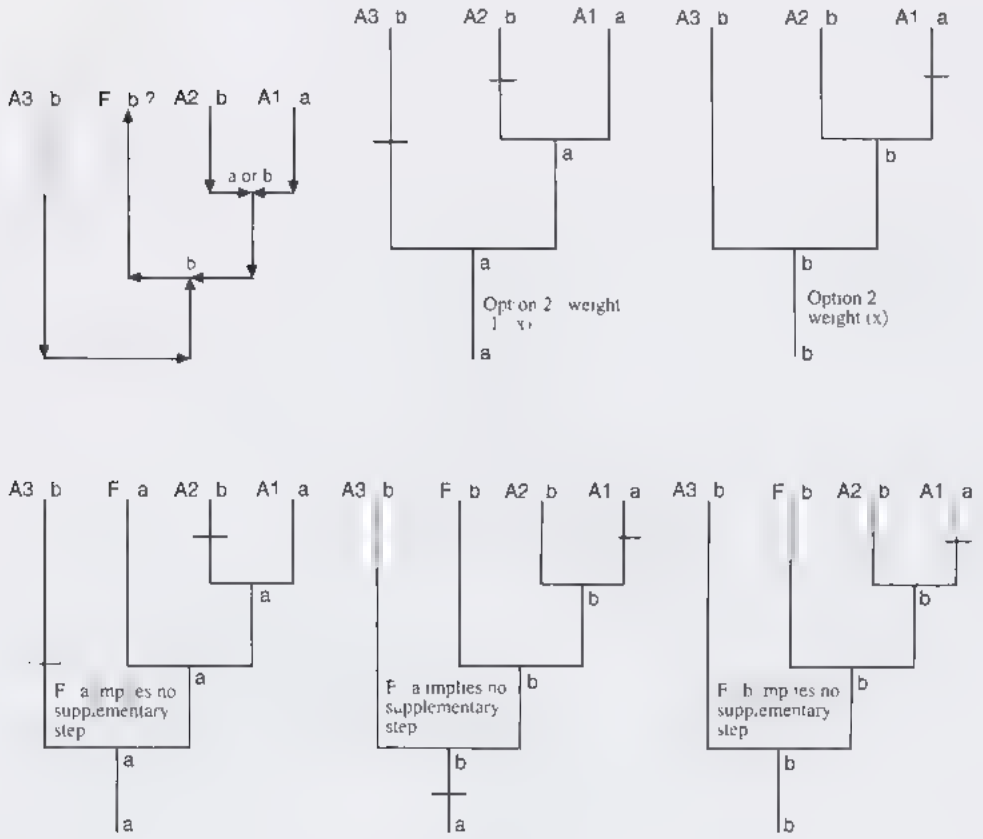

FIG. 5. — p(F . a) = 1/3

More generally

$$2/3 \leq p(F1 \cdot a)(x) \leq 1 \text{ and } 1/3 \leq p(F2 \cdot a)(x) \leq 1$$

because $0 \leq x \leq 1$; p (F1 : a) and p (F2 a) are decreasing functions of x.

Thus, in this case, the probabilities are not the same, because of the "attraction" of the low branch Furthermore, all the probabilities decrease with the number of F-taxa between A2 and A1 This result is congruent with the assumption that the possibilities of the displacements of the positions of the transformations within the tree increase with the number of F-taxa between A1 and A2

8) With n F-taxa in the same situation as in Figure 7, probabilistic method gives [option (1)]

$$p(F1 : a) = (n+1)/(n+2),\ p(F2\ a) = n/(n+2),\ \ldots,\ p(Fi \cdot a) = (n+2\ i)/(n+2),\ \ldots, \text{ and}$$

$$p(Fn \cdot a) = 2/(n+2)$$

If the rank "i" of a F-taxon is supposed constant, the probabilities p (Fi a) increase with n For example, p (F1 a) increases with n Nevertheless, the possibilities of having the state "b" for some of these F-taxa increase with the number of taxa between A2 and A1, even if the "distance" (number of steps) between A1 and A2 do not increase

Source MNHN, Paris

The better possible schema is that with two known taxa in the state "a" and an out-group in the state "b".

Multistate attribute

Definition In many cases, the attributes have more than two states (multistate attribute or character, especially for the climatic analysis) For example, in a "temperature" analysis based on the scale of DAVID *et al* (1983), the attribute shows five states [Icy - Cold - Temperate - Hot - Torrid] For each state, an arbitrary value can be attributed, ranging from "a" to "e" Several scenarios can be envisaged

If the various states seem to correspond to a gradual process, it is possible to envisage the hypothesis of their polarization following a gradation without any "jump" between two successive states, from an extreme state to an other, *i.e.* [- a → b › c › d › e +] or [- e → d › c → b → a +] for a "temperature" analysis, or from an intermediate state towards the extreme states. [+ e ← d ← c (-) › b → a +] for example

It is possible to deny any gradual process *a priori*, but to still suppose the existence of an evolution from a plesiomorphic state towards one or several apomorphic states, with possible "jumps" over some states For example, a possible polarization would be [- a → b → c → d → e +] In that case, the number of possible polarization's quickly increases There are nine possibilities for three states but 64 possibilities for four states of a character

Theoretical procedure If it is possible to define the more probable scenario for the attribute after the analysis of the known taxa following the parsimony method the scenario that implies the weaker quantity of homoplasies or steps BRYANT & RUSSEL's method can be applied using similar processes of inference of the situations at the nodes as for bipolar characters Probabilistic method can also be used with the following change the minimal scenarios (with the lowest number of steps) that explain the known distribution of the attribute (excluding the F-taxon) are reconstructed for all the situations of the root "root a", "root b", "root c", etc

They can be considered as equally probable, even if one can imply more steps than the other [option (1)] The option (2) gives different weights x_a, x_b, x_c, etc , to the various situations of the roots, with the $\sum_i (x_i) = 1$ As the various values of the x_i are unknown, this option is practically inapplicable Next step is to re-included the F-taxon in the tree, then are only accepted the situations where the alleged state for F can be reconstructed without adding supplementary homoplasies or steps in the tree ("favorable cases"), in order to keep the same minimal lengths for the new trees This method implies that we deny any gradual process *a priori* (*contra* first scenario as above)

Application For an attribute X with three states "a", "b" and "c" within a group of taxa, including the taxon F (Fig 8)

BRYANT & RUSSEL's method gives the inference of "a or b or c" for F, even if the situations for A1, A2, and A3 are permuted

Probabilistic method [with option (1)] leads to a similar conclusion but it is more precise because it will be affected by permutations of the situations for A1, A2 and A3, for examples

If A1 is "c", A2 is "b" and A3 is "a" (Fig 8), then p (F c) 2/6 1/3, p (F b) = 3/6 - 1/2 and p (F : a) - 1/6

Source MNHN Paris

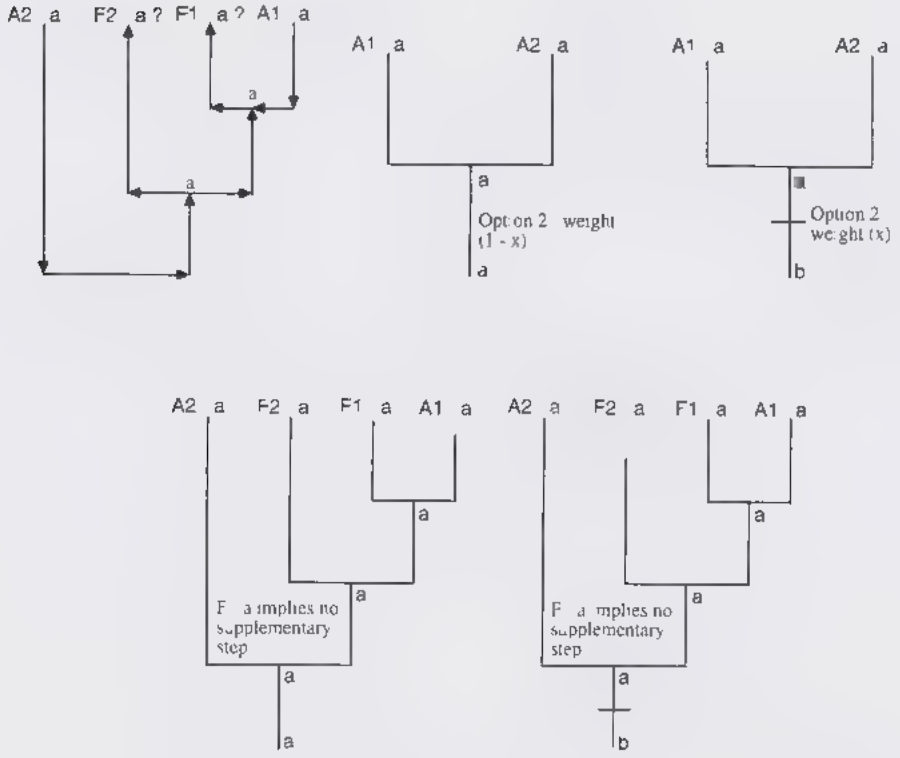

FIG. 6. — p(F1 a) – p(F a) = 1 , other situations imply supplementary steps

If A1 is "b", A2 is "c" and A3 is "a", then p (F b) − 2/6 = 1/3, p (F c) – 3/6 1/2 and p (F : a) = 1/6

Both these results are congruent with the position of sister-groups between A1 and F Similar results can be obtained within each case of permutation between A1, A2 and A3

In the situation of Figure 9, the results of the two methods slightly differ BRYANT & RUSSEL's method gives the inference "b" for F but probabilistic method [with option (1)] gives p (F : b) = 3/4 and p (F : c) = 1/4, similarly to the situation of Figure 5.

Conclusion

It is possible to calculate a probability law for each character or attribute which is unknown for a taxon included in a phylogeny These calculations give the maximal estimation of the probability for the inference, because the additions of steps due to the presence of the F-taxa are rejected, but they could have happened These two different methods of inference explain the weakness of the theory of actualism concerning the ancient palaeobiotas It is not directly the antiquity of the fossil taxa which renders less probable the inference of the attributes to the fossil, but if a fossil is older than another one, it has more "chance" to be only the sister-group of Recent taxa, thus, it only provides information of low probability The scale of measure of the reliability of the inference is not directly temporal but phylogenetic, thus it is not directly related with the time factor

Source MNHN Paris

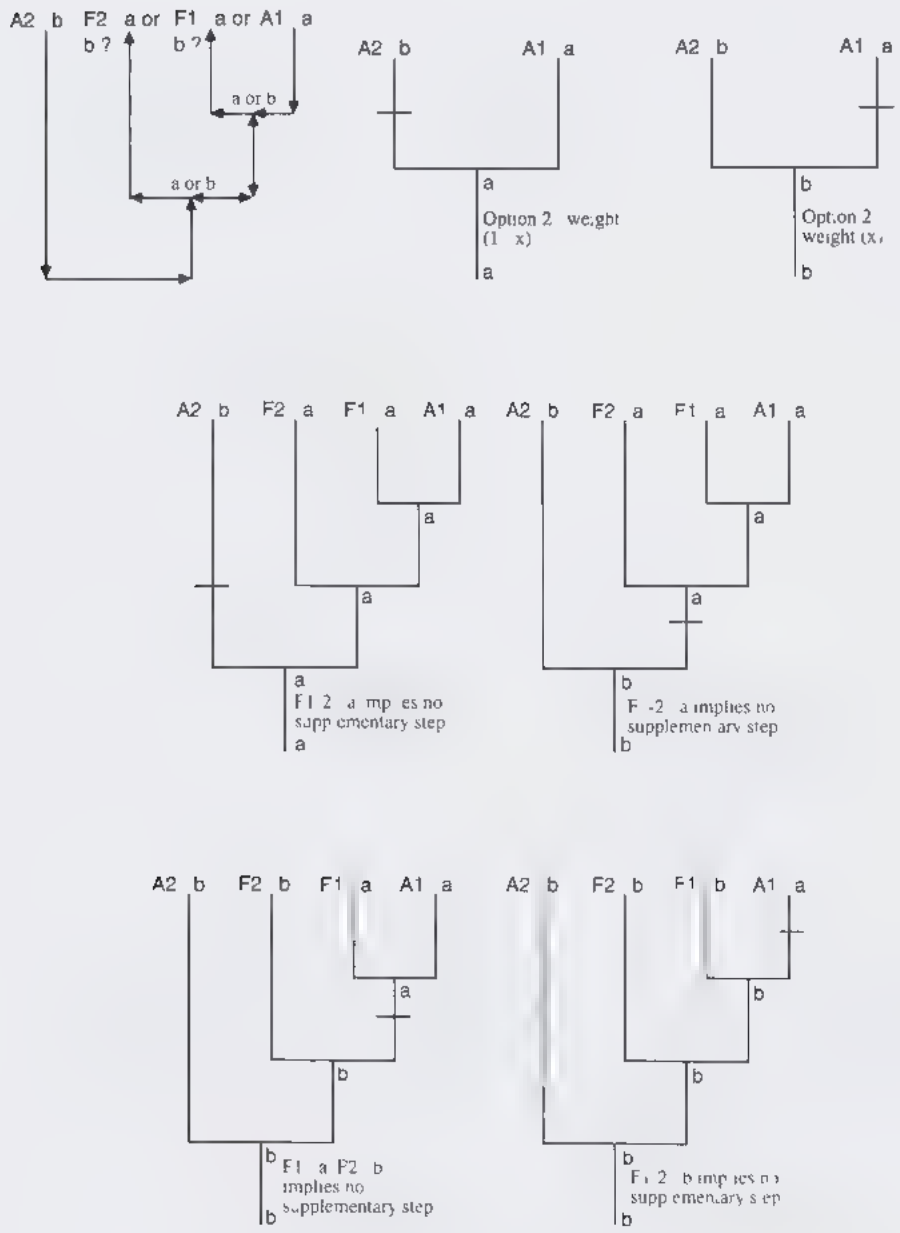

FIG. 7 — p(F1 . a) = 3/4 , p(F2 . a) = 1/2 , other situations also imply supplementary steps

Application : palaeoclimatic and palaeoenvironmental phylogeny

Procedure All the palaeoclimatological studies based on the fossil data use the comparison between fossil taxa and their "nearest" Recent relatives. More especially, palaeoclimatic studies of the Quaternary are now based on the elaborate method of the "Mutual Climatic Range" or

Source: MNHN, Paris

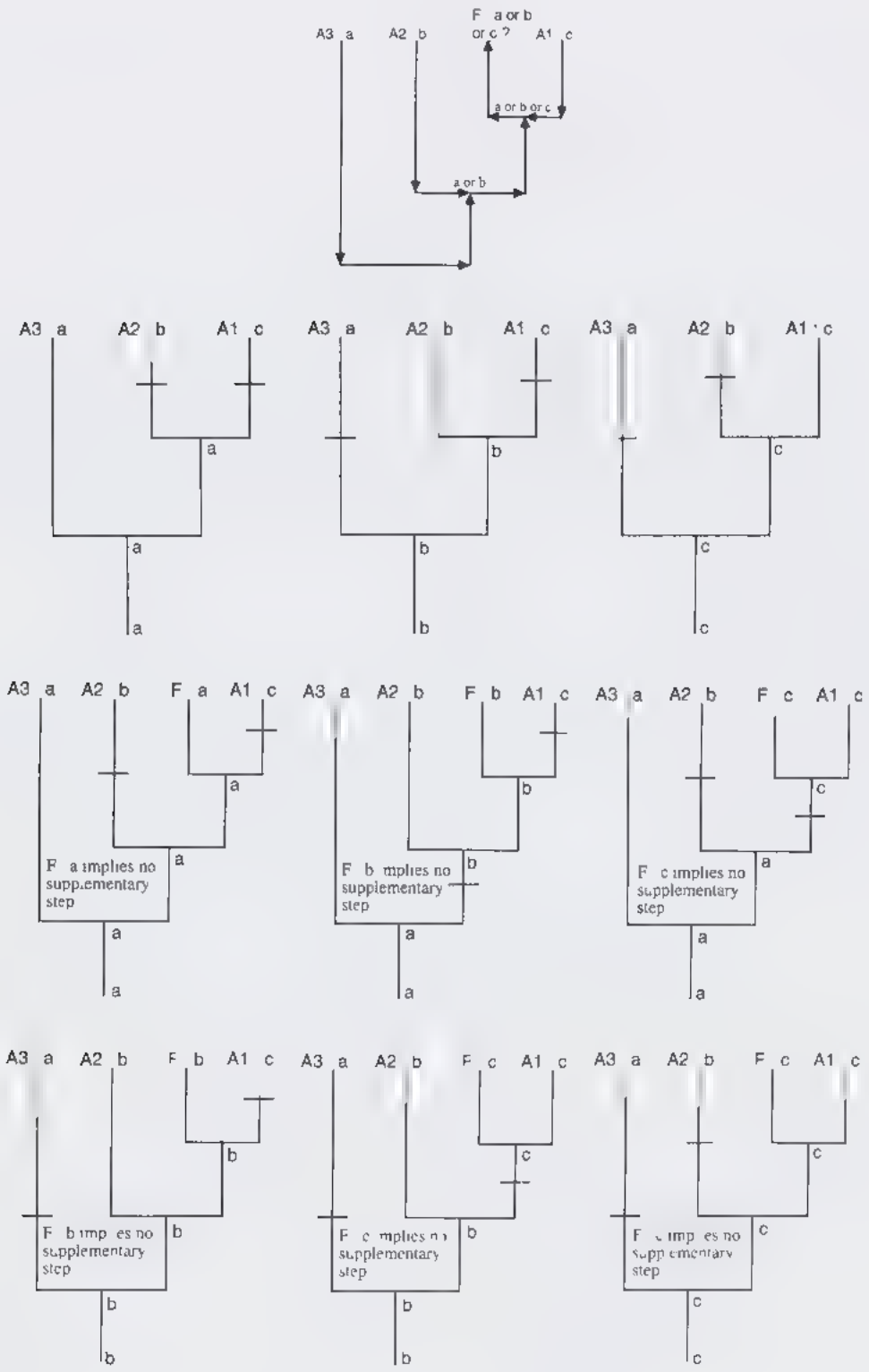

FIG 8 — p(F a) = 1/6 , p(F b) = 1/3 , p(F c) = 1/2

Source MNHN, Paris

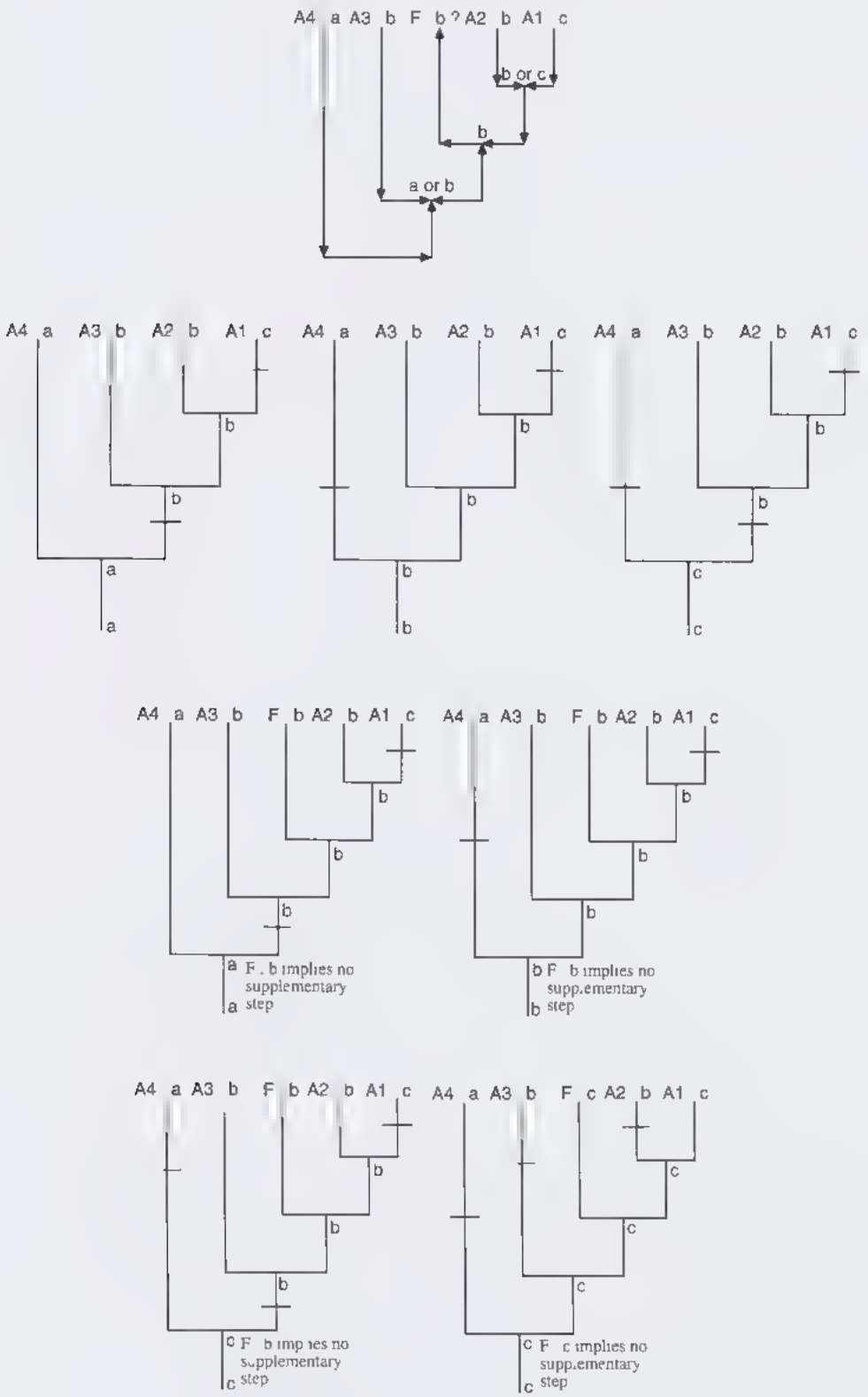

FIG 9 p(F a) = 0 , p(F b) = 3/4 , p(F c) = 1/4

Source MNHN Paris

"MCR" of ATKINSON *et al.* (1987) It establishes a theoretical palaeoclimate which corresponds to the "mutual intersection of the tolerance range" of the various subfossil taxa present in the studied deposit This method implies that the climatic tolerances of the studied species did not vary through the time, the (sub)fossil taxa can be identified as being living species, the various climatic tolerances of the (sub)fossil taxa have an intersection, all the informations share the same weight, *a priori* This method becomes difficult to apply for strictly fossil taxa but the method of phylogenetic inference can help The complex nature of a climate implies precise definitions of the used parameters For example, DAVID *et al* (1983) defined a (palaeo)climate after the combination of three types of climatic factors [glacial - cold - temperate - hot - torrid], [arid - dry - sub humid - humid] et [stable - alternative] AXELROD (1992) proposed another climatic scale based on the definition of the "equable climate" characterized by a mean annual temperature of 14°C and a mean annual variation of 0°C Whatever the scale, it is necessary to distinguish the different (palaeo)climates using discrete scales, in order to consider the data as characters (or attributes) which can be tested by a phylogenetic analysis

The theoretical method is derived from BRYANI & RUSSELL (*loc. cit.* 409, Fig 1) with the two following steps

As a first step, an analysis of inference, taxon after taxon, of the characters or attributes of unknown state Each fossil taxon is integrated, when possible, in a phylogenetic analysis based on the present morphological characters, but not based on the attributes which shall be studied after For each climatic attribute (temperature, humidity, stability) and each taxon, the probability law of the attribute is established A study of correlation [structure - function] based on the preserved characters of each fossil is to be made in parallel with the study of phylogenetic inference The conclusions of the two phylogenetic and extrapolated procedures are compared If the results are congruent, the law of probability of the concerned attribute is taken up for the taxon Otherwise, the taxon is considered as doubtful and is not used for the following step Its phylogenetic placement is reexamined and its law of probability is recalculated, and compared again to the results of the study of correlation [structure - function]

As a second step, an analysis of inference of the states of the attributes for the studied palaeoenvironment is based on all the inferences established during the first step By putting together all the data for all the taxa (F_j), for each attribute X, is calculated a series of coefficients $[P_i (X)]_i$ The "i" correspond to the states of the attribute X

$$\text{Each } P_i (X) = \sum_j [p (X . i \text{ for } F_j)]$$

with the F_j corresponding to all the present taxa

A law of probability L (X) of the attribute X for the concerned palaeobiota, can be established On the basis of this law of probability, a mean value E (X) for the attribute can be calculated

$$E (X) = P_i (X) / \sum_i [P_i (X)]$$

The results of the phylogenetic analysis are to be tested, when possible, by independent data gathered with a direct physical analysis (analysis of the Oxygen isotopes, or of Deuterium/Hydrogen, etc , MILLER *et al* , 1988) Similarly, the results of phylogenetic biogeography are to be tested using the independent geological data (NELSON, 1985) If the results are congruent,

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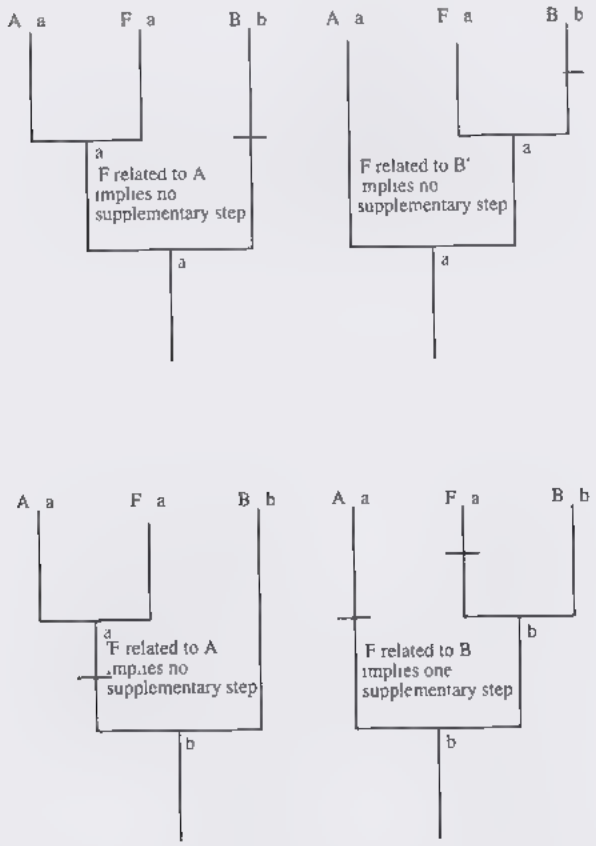

FIG 10 — p(F is related to A) = 2/3

they are considered as probable Otherwise, the law of probability of the attributes can be verified for all the taxa and new data are to be found out before solving the problem

Examples Within this theory, for a study of palaeotemperature T (with the rate [glacial "a" - cold . "b" - temperate . "c" - hot · "d" - torrid "e"]

If we consider a taxon F has the following law of probability:

$$p(T \cdot \text{“b” for F}) = 3/4,\ p(T : \text{“c” for F}) = 1/4$$

$$\text{and } p(T \text{ “a” for F}) = 0 = p(T : \text{“d or e” for F})]$$

(corresponding to the situation in Fig 9).

Then, F has more weight for the calculation of the law of probability of the global palaeotemperature of the palaeobiota than a taxon F' with the following law of probability for the palaeotemperature.

$$p(T \cdot \text{“b” for F'}) = 2/6$$

$$\text{and } p(T \cdot \text{“c” for F'}) = 3/6,\ p(T \text{ “a” for F'}) = 1/6,\ p(T : \text{“d or e” for F'}) = 0$$

(corresponding to the situation in Fig 8)

This method of weighting gives a greater importance to the taxa which correspond to highly specialized climatic conditions It allows a less empirical evaluation of the weights

Source MNHN Paris

Applications concerning "Mutual Climatic Range" The same method of weighting can be applied in the subfossil record as a modification of the "Mutual Climatic Range", by giving a more important weight to the taxa specialized to only one type of biota or of climate A Recent (or subfossil) taxon which is present under climates for which the temperature ranges from type "5" to type "3" directly gives, without inference analysis, the following law of probability

$$p(T < 3) = 0,\ p(T \cdot 3) \quad p(T : 4) = p(T : 5) = 1/3$$

Unlikely, a living taxon present under a climate for which the temperature is of type "5" gives the law of probability

$$p(T < 5) = 0 \text{ and } p(T \cdot 5) = 1$$

The same method is to be applied to all the taxa The following analysis is that of the precedent step

Applications concerning non inference of an attribute allochtony If the law of probability for an attribute of a F-taxon does not correspond to the general law of probability of the biota, its climatic or environmental constraints could have changed relatively to the nearest relative taxa or the concerned F-taxon is allochtonous for the concerned palaeobiota

Problem of the living fossils or relic taxa (sensu DELAMARE-DEBOUTEVILLE & BOTOSANEANU, 1970) Theoretical problem the characters or attributes which are only present in one Recent taxon A cannot be easily inferred in the fossil record, whether they are autapomorphies of A or symplesiomorphies of the group including A For all the possible hypothesis of weighting of the homoplasies, the probability p (F a) – p (F b) – 1 2, situation of simple information of a sister-group relationship between F and a Recent taxon A, and the inference or the non-inference of the attribute X from A on F are equally probable This situation occurs specially in the cases of relic taxa, which are the only Recent representatives of fossil groups They are poorly informative in the inference of their own characters or attributes The relic species are often located in peculiar "refuges" which are very different of the palaeobiotas of their nearest fossil relatives, as already noticed for some marine taxa which are supposed to have "migrated" from shallow water zones towards deep water zones during the Palaeozoic or Mesozoic (the Mollusk *Neopilina galatheae* Lemche, 1958 or the Crinoids for examples, DELAMARE-DEBOUTEVILLE & BOTOSANEANU, 1970)

As a first example, I can show that the Isoptera Mastotermitidae are poor palaeoclimatic indicators The Recent termite *Mastotermes darwiniensis* (sole living representative of the family Mastotermitidae) lives under the very peculiar climate and biota of the savanna ("bush") of Northern Australia (GAY & CALABY, 1965 396), but seems to be absent in the evergreen forest (EMERSON, 1965 27) This insect is an excellent climatic and environmental indicator for the present days Contrary to the hypothesis of NEL & PAICHELER (1993), the direct inference in the past of all these climatic and environmental data, using the presence of fossil *Mastotermes* spp in Cenozoic palaeoenvironments, has a low probability of 0 5 because these fossils have (in the best case) only relationships of sister species with the Recent taxon Thus, it is impossible to say that these fossils lived in palaeobiotas similar to that of *M. darwiniensis* The two fossil genera *Blattotermes* and *Spargotermes*, other known Mastotermitidae, give no more palaeoclimatic and palaeoenvironmental informations because they are, in the best case, only the sister genera of *Mastotermes* The Kalotermitidae (and other isopteran families) live under temperate, hot or

Source MNHN Paris

torrid climates and the Mastotermitidae live under hot or torrid climates Either if we consider that the Mastotermitidae are really the sister-group of the Kalotermitidae (ROONWAL, 1985), or the sister-group of all other isopteran families (KAMBHAMPATI *et al* , 1996), inference analysis of the attribute "temperature", with the two states "temperate" ("a") and "hot or torrid" ("b"), shows that the probability for the fossil Mastotermitidae to have lived under "hot or torrid" temperatures p (F "b") 2/3 and the contrary probability p (F "a") = 1/3 (situation of figure 5) In a different way, BRYANT & RUSSEL'S method gives the inference of the state "b" for F and appears less precise. This example shows that only a small part of the information can be inferred in the fossil record

As a second example, the Brachiopoda, Lingulidae show a case of ineffectiveness of the inference method These animals usually live on the light bottom of the tide zone but PAINE (1970) indicates a species (*Lingula albida*) living in deep water The phylogenetic inference of the former biota in the fossil record, as proposed by GALL (1971 24) for the Triassic of Vosges (France), is difficult to establish because of the lack of phylogenetic analysis of the group which integrates the fossil taxa The Triassic, Devonian and Ordovician Lingulidae are attributed to the genus *Lingula s.l.*, and their real relationships with the Recent genera *Glottidia* and *Lingula* remain uncertain The probability they had lived in deep water is equal to the probability they had lived in shallow water Some more precise informations concerning the substrate on which these animals did live can be found out after the morphological and physical analysis of the fossil shells and ancient substrate (PAINE, 1970), the phylogenetic data being useless

INFERENCE OF THE POSITION OF A TAXON

Theoretical procedure

The available characters in the fossil record are frequently highly homoplastic (for example some of the odonatan venational structures) Thus, it is difficult to attribute a fossil taxon to a precise group on the sole basis of these ambiguous characters Nevertheless, it is possible to estimate a probability for the event [the taxon is related to a group rather than to another one], following a probabilistic method similar to the precedent This method can be applied to a taxon F clearly related to two possible groups A and B, but which does not share any clear synapomorphy with A or B, to the exclusion of one of the two groups The two events [F is related to A] and [F is related to B] are opposite

Two hypotheses of scenario can be made

First, with the supplementary hypothesis that, for each concerned character, the probabilities for the additions of new steps are equal to zero and that the other possible situations of polarity are equally probable, the probability of the events [F is related to A] and [F is related to B] can be calculated with the quotient of the number of favorable cases by the total number of possible cases

Second, a weight p (arbitrary, medium or maximal) can be given to the simple addition of a new step ($0 < p < 1$ if p is calculated as a percentage or a rate of homoplasy) The non-addition of a step will have a weight q 1 – p Furthermore, [p – 0] corresponds to the probability zero for the addition of a new step

X is a character that is supposed to have two states "a" and "b" As the polarity of the character is completely unknown, we consider that it can equally be in the states "a or b" in the root of the tree Then, in the simple tree made with the two taxa A and B, the minimal scenarios

Source MNHN Paris

(with the lowest number of steps) that explain the known distribution of the attribute (excluding the F-taxon) are reconstructed for the two situations "root a" and "root b", and considered as equally probable, even if one can imply more steps than the other After, the F-taxon is re-included in the partial tree of the taxa A and B, then are only accepted the situations where the alleged state for F can be reconstructed without adding supplementary homoplasies or steps in the tree ("favorable cases"), in order to keep the same minimal lengths for the new trees

Examples

If the state "a" is shared by F, A and B, no homoplastic situation appears whether we consider X as an apomorphy or plesiomorphy The character "a" is not informative

$$p([F \text{ is related to } A]) = p([F \text{ is related to } B]) = 1/2$$

If the state "a" is present in F and A but absent in B, the polarity of which is ambiguous (presence of several homoplastic situations concerning the character in the phylogenetic analysis) (Fig 10) If p = 0, there are only three "possible" situations, with two "favorable" for the hypotheses [F is related to A], the universe of the possibilities is {(root a , F is related to A) noticed (root a , F-A) , (root a , F is related to B) noticed (root a , F-B) , (root b , F is related to A) noticed (root b , F-A)} F is more probably to related to A than B

Probabilities are

$$p(F \text{ is related to } A) = 2/3 \text{ and } p([F \text{ is related to } B]) = 1/3$$

If we suppose that p for the added steps is not nil, there are four "possible" situations with different weight The universe of the possibilities is {(root a , F-A) with a weight q , (root a , F-B), q , (root b ; F-A), q ; (root b ; F-B) p}.

The probability are

$$p([F \text{ is related to } A]) = (2q) / (3q + p) \text{ or}$$
$$p([F \text{ is related to } A]) = (2q) / (2q + 1)$$

As there are two favorable cases with the weight q against four possible cases, including three cases with a weight q and one with the weight p If p 0, we find again q = 1 and the precedent probability For all the possible values of p, the "best" possible value of the probability is that corresponding to p 0, because $2/3 > (2q) / (3q + p)$ for all values of p

$$(2q) / (3q + p) = 2(1 - p) / (3(1 - p) + p) - 2(1 - p) / (3 - 2p) = (2 - 2p) / (3 - 2p)$$
$$\text{and } (2 - 2p) / (3 - 2p) < 2/3 \text{ if } (2 - 2p) \times 3 < (3 - 2p) \times 2 \text{ if } 6 - 6p < 6 - 4p$$
$$\text{if } 6p > 4p \text{ (with } 0 < p < 1)$$

If we add a character X2, also of uncertain polarity, independent of X1 and with state "a" shared by F and A and state "b" for B, the universe of the possible events is the product of the universes of the possibilities corresponding to X1 and to X2.

If the weight p = 0 for the homoplasies, the universe is {(root "a" for X1, root "a" for X2 and F is related to A) [or with an abbreviated notation (a X1, a X2 , F-A)] , (a X1, b X2 , F-A) , (b X1, a X2 , F-A) , (b X1, b X2 , F-A) , (a X1, a X2 , F-B)} All the cases of the type (one of the roots is in the state "b" and [F is related to B]) imply an homoplasy and are not counted There are five possible cases with four favorable to [F is related to A] Consequently, p ([F is related to A]) 4/5 and p ([F is related to B]) = 1/5 The probability for (F is related to A) increases

Source MNHN, Paris

If the weight p is not nil, the homoplasies are counted and the universe becomes {(a X1, a X2 , F-A), weight q^2 , (a X1, b X2 , F-A), q^2 , (b X1, a X2 , F-A), q^2, (b X1, b X2 , F-A), q^2, (a X1, a X2 , F-B), q^2, (b X1, a X2 , F-B), pq , (a X1, b X2 , F-B), pq , (b X1, b X2 , F-B), p^2} There are four cases favorable to "F-A" with a weight q^2 There are also four cases unfavorable to "F-A", one with a weight p^2, two with a weight pq and one with a weight q^2

$$p([F \text{ is related to } A]) \cdot (4q^2) / (5q^2 + 2pq + p^2) = (4q^2) / [4q^2 + (p + q)^2]$$

As (p + q) = 1, I find

$$p([F \text{ is related to } A]) = (4q^2) / [4q^2 + 1]$$

If p = 0, q 1 we find again p ([F is related to A]) 4/5 The best possible value of p ([F is related to A]) occurs when p = 0 (the homoplasies are impossible) because $(4q^2) / [4q^2 + 1] < 4/5$ is equivalent to $4q^2 < 4$ or $q^2 < 1$ Otherwise, if p is different of 0, the probability p ([F is related to A]) varies between 4/5 and 0.

In the case of n characters X1, X2, X3, X4, , Xi, , Xn which are all in the same situation (with an uncertain polarity, independent, with state "a" shared by F and A and state "b" for B),

If p 0, the universe of the possibilities is of cardinal $(2^n + 1)$, with (2^n) events in favor of [F is related to A], thus

$$p([F \text{ is related to } A]) = (2^n) / (2^n + 1) \text{ and } p([F \text{ is related to } B]) \quad 1 / (2^n + 1)$$

If p is not nil, the cardinal of the universe increases to the value $2 \times 2^n \quad 2^{n+1}$

2^n events are favorable to [F is related to A] with a weight q^n, the other events correspond to [F is related to B], with one having the same weight q^n, n events have the weight pq^{n-1}, $(n!) / [2! (n - 2)!]$ events have the weight $p^2 q^{n-2}$, $(n!) / [3! (n - 3)!]$ events have the weight $p^3 q^{n-3}$, etc., and one event has the weight p^n

The probability p ([F is related to A]) = $(2^n q^n) / [(2^n + 1)q^n + npq^{n-1} + \{(n!)/[2!(n 2)!]\}p^2q^{n-2} + \{(n!)/[3!(n 3)!]\}p^3q^{n-3} + + p^n]$ There is an usual remarkable identity in the denominator thus

$$p([F \text{ is related to } A]) = (2^n q^n) / [(2^n q^n + 1)$$

This formula generalizes the preceding ones Furthermore, if p = 0 and q = 1, we find again the formula $(2^n) / (2^n + 1)$ For all cases, the maximal value of p ([F is related to A]) is equal to $(2^n) / (2^n + 1)$, when p varies from 0 to 1.

If there is a character X (bipolar, a or b) with the state "a" shared by A and F but not by B and one character Y (bipolar, a or b) with the state "b" shared by B and F but not by A (situation symmetrical of Figure 10)

In the case of a weight p = 0 for the added steps, the universe of the possibilities is {(a X, a Y , F-A) , (b X, a Y , F-A) , (a X, b Y , F-B) , (a . X, a Y , F-B)} , there are four events with two favorable to [F is related to A] and 2 are favorable to [F is related to B], p ([F is related to B]) ⁻ p ([F is related to A] = 2/4 = 1/2.

If p is not nil for the added steps, the universe becomes {(a X, a Y , F-A), q^2 , (b X, a Y , F-A), q^2 , (a X, b Y , F-B), q^2 , (a X, a Y , F-B), q^2 , (a . X, b . Y , F-A), pq , (b X, a Y , F-A), pq , (b X, a Y , F-B), pq , (a X, b Y , F-B), pq} p ([F is related to A]) = $(2q^2 + 2pq) / (2q^2 + 2pq + 2q^2 + 2pq) = 1/2$

In the two hypothesis, the two informations of X and Y "neutralize" each other

Source MNHN, Paris

Generalization

If we have n characters of the type X with the state "a" in common to F and A and m characters of the type Y with the state "b" in common to F and B

In the case of a weight p = 0 for the added steps, the universe holds $[(2^n) + (2^m)]$ events which are distributed into (n+ m) -upsets of two types

(2^n) events of the type (a : Y1, a . Y2, ..., a : Yi, ..., a : Ym, a or b : X1, a or b : X2, ..., a or b · Xi,..., a or b . Xn, F-A);

(2^m) events of the type (a or b : Y1,..., a or b · Yi, ..., a or b : Ym, a : X1, ..., a : Xi, ..., a : Xn, F-B)

Consequently,

$$p([F \text{ is related to } A]) = (2^n) / [2^n + 2^m]$$

$$p([F \text{ is related to } B]) = (2^m) / [(2^n) + (2^m)]$$

In the case of a weight p for added steps, then·

$$p([F \text{ is related to } A]) \quad 2^n q^n [q^m + mpq^{m-1} + \quad + p^m] / 2^n q^n [q^m + mpq^{m-1} + \quad + p^m] + 2^m q^m [q^n + npq^{n-1} + \ldots + p^n],$$

then

$$p([F \text{ is related to } A]) = 2^n q^n [p + q]^m / (2^n q^n [p + q]^m + 2^m q^m [p + q]^n)$$

as $(p + q) = 1$, then·

$$p([F \text{ is related to } A]) = 2^n q^n / (2^n q^n + 2^m q^m)$$

This formula generalizes and replaces all the precedent ones

p = 0 and q - 1 give again p ([F is related to A]) $(2^n) . [2^n + 2^m]$ which is the maximal possible value when p varies from 0 to 1 If m n, p ([F is related to A]) 1/2 The contradictory informations "neutralize" each other

Furthermore, p ([F is related to A]) - 1/2 (for all the values of m and n) if q 1 2 *i.e.* if the weight p of the addition of steps = 1/2 Even if there are distinctly more characters in favor of a relation with A rather than with B (n >> m), if the probability that all these characters implies additions of new steps is too important, it is impossible to decide

BECHLY *et al* (1997) apply this method to the peculiar case of the Lower Cretaceous English Zygoptera *Cretacoenagrion* (taxon of uncertain position because of the lack of information) There is a maximal probability of 4/5 for the event [*Cretacoenagrion* is related to the Lestoidea rather than to the Coenagrionoidea] but it is still impossible to state positively that it is a Lestoidea

If the number of shared characters between F and A but not by B increases, the probability of the event [F and A are related] increases This result is congruent with an intuitive approach of the problem This method does not prove that F is really related with A and would not replace the cladistic method based on the principle of the fundamental importance of the synapomorphies for the determination of the relationships between the taxa This method gives an estimate of the probability p ([F is related to A]) but the calculation of the exact value of this probability depends on the determination of the rate p of the homoplasies The result can greatly vary with the value of p. A probability, even very high, is not a proof

Source MNHN Paris

CONCLUSION

Although these methods of probabilistic inferences could appear not very easy to use, they have the advantage of quantifying the possibilities of transferring Recent biological and environmental data to (sub)fossil taxa. Thus, they limit and refine possibilities of global transferring of actualism. Quantification of inferred palaeoclimatic data allows establishments of more precise palaeoclimatic hypotheses, susceptible of being tested by physical analysis. Comparisons between palaeoclimatic hypotheses of different palaeobiotas shall be easier to attempt because these hypotheses are based on the same method. The probabilistic inferences of taxa positions cannot replace phylogenetic analyses but they are better than subjective and not quantified hypotheses.

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The Origin of Hexapoda: a Developmental Genetic Scenario

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ABSTRACT

The similarities and differences in the body plans of Crustacea and Hexapoda are discussed and analyzed Based on phylogenetics and comparative developmental genetics, an evolutionary scenario is proposed on the origin of Hexapoda Basically, it is assumed that Hexapoda would derive from an ancestor bearing crustacean-like characters Changes in the pattern of expression of *Hox* genes would be correlated to the observed changes in body plans Secondarily, the "master gene" *Distalless*, required in limb formation, would have passed under the control of the *Hox* genes This change would have produced the reduction of leg pairs to a number of three, and would be fixed by terrestrial life The interest of the interplay of comparative developmental genetics and of phylogenetics is stressed, the former being able to propose realistic evolutionary scenarios at the genetic level, the second to critically test them

RÉSUMÉ

L'origine des Hexapodes : un scenario de génétique du développement

Le présent article décrit un scénario concernant l'origine évolutive des Hexapodes Sur la base de phylogenies récemment proposées à partir de données tant moléculaires que morphologiques on suppose que les Hexapodes dériveraient d'un ancêtre de type Crustacé Dans la lignée conduisant aux Hexapodes, un changement se serait produit dans le profil d'expression des gènes *Hox* Ce changement rend compte des différences de plan du corps entre Crustacés et Hexapodes Dans un second temps, le gène *Distalless*, responsable de la morphogenèse des appendices, serait passé sous contrôle des gènes homéotiques Cela aurait provoqué la réduction à trois du nombre de paires de pattes Ce changement aurait été fixé par l'habitat terrestre des Hexapodes La rencontre nécessaire de la génétique du développement et de l'analyse phylogénétique est soulignée, la première permettant de proposer des scénarios d'évolution vraisemblables au niveau génique la seconde permettant de tester la validité de ces scénarios particuliers

DEVELOPMENTAL GENETICS AND EVOLUTIONARY BIOLOGY

Until the birth of the "Synthetic theory", genetics (Mendelism) and evolutionary biology (Darwinism) developed separately and sometimes were in conflict Similarly, for a time, genetics and embryology were quite separate fields This time is now behind us, as shown by the recent recognition of developmental genetics by the Swedish Academy, awarding the Nobel Prize to the founders of the developmental genetics of *Drosophila*, E LEWIS, C NUSSLEIN-VOLHARD and E WIESCHAUS (see DEUTSCH *et al.*, 1995) In parallel, developmental biologists today direct their research to the molecular and genetic mechanisms of development However, for epistemological

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and also obvious practical reasons, up to now, both developmental biology and genetics have focused on a small number of model organisms

The time is now ripe for a new field comparative molecular developmental genetics, at the crossroads of evolutionary biology, genetics and embryology (LAWRENCE & MORATA, 1994) The growth of this new field is indeed currently boosted by i) the discovery of the homeobox twelve years ago and the widespread distribution of homeobox genes (GEHRING, 1994) which opened our minds to the idea of the evolutionary conservation of developmental genes across the Metazoan kingdom, and ii) the extraordinary power of newly developed tools in molecular biology

Developmental genetic information can be of high phylogenetic value It yields new available characters, that can be integrated like other characters in phylogenetic analyses In my opinion, the type of data generated by this new discipline deserves more particular attention, due to their dual quality of being both genetic *and* developmental As genetic, because the genome is the place where the whole history of life is engraved, as a consequence of the Darwinian principle of "descent with modification", which must be the basis of all phylogenies As developmental, since embryologic and larval characters may be of higher taxonomic (*i.e.* phylogenetic) value than adult ones, according to Darwin himself, (DARWIN, 1859), as he experienced in his study of the crustacean cirripedes Without taking for granted GARSTANG's sentence that "*ontogeny creates phylogeny*" (for a comment see DEVILLERS & TINTANT, 1996), it should be stressed that evolutionary radiations in the Metazoa correlate with changes in the body plan, which is the result of genetically controlled developmental processes.

The interface of developmental biology and phylogenetics could be the promise of a "New synthesis" in the theory of evolution (GILBERT, 1991).

THE PURPOSE OF THE PRESENT ARTICLE

Here I emphasize the juxtaposition of developmental biology and phylogenetics by proposing an evolutionary scenario of the origin of Hexapoda based mainly on our current, and I must confess, still scarce, knowledge of the developmental genetics of crustaceans and insects

So doing, I include myself in the tradition of developmental genetics M AKAM (AKAM *et al.*, 1988) synthetized the hypotheses of LEWIS (1978) on *Hox* gene evolution and the developmental model proposed by RAFF & KAUFMAN (1983), itself derived from SNODGRASS, and proposed a scenario in which insects derived from an annelid-like ancestor by progressive steps These included onychophoran- and myriapod-like states, correlated with an increase in the number of *Hox* (homeotic) genes (see below for more details on *Hox* genes)

Given the improvements of our knowledge in both phylogenetics, which now rejects any close relationship between annelids and arthropods, (EERNISSE *et al.*, 1992, KIM *et al* , 1996) and of comparative molecular developmental genetics, which has shown that a large gene complex was likely already present at the origin of arthropods (see below and Fig 1), the main features of AKAM's 1988 scenario are presently unvalidated Nonetheless, this model has been quite useful in two ways summarizing the current knowledge of different scientific fields and stimulating new experimental research

Source MNHN, Paris

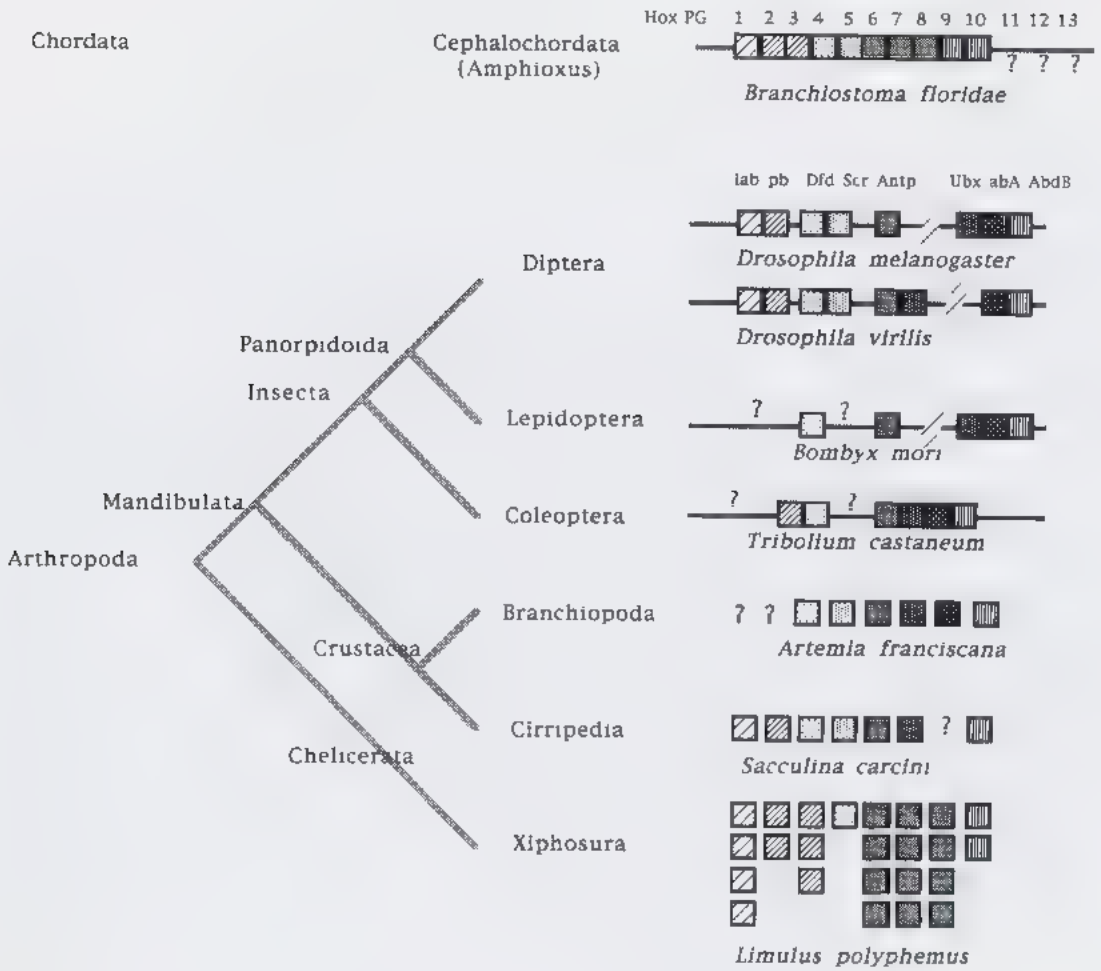

FIG 1 The evolution of *Hox* genes **Right** The *Hox* genes of a selection of arthropod species are represented, after available data in the literature Each *Hox* gene is represented by a square of a distinct color Gene names are given above the squares for *Drosophila melanogaster* Genes of the same color in different species are assumed to be orthologous A question mark means that the gene of interest has not been reported to date which does not prove that it is not present The squares are lined on a bar when genetic linkage has been demonstrated by genetic and/or molecular evidence A split in this bar indicates a split of the *Hox* complex at this position The absence of a bar in a given species indicates that up to now there is no direct experimental evidence for the grouping of the *Hox* genes in a complex in this case For sake of clarity non homeotic genes physically present within the gene complexes of *Drosophila* are not represented **Left** A likely phylogenetic tree of the arthropod species represented is drawn **Top** For comparison, the *Hox* gene complex of the *Amphioxus*, probably close to the ancestral chordate one, is drawn (GARCIA-FERNANDEZ & HOLLAND, 1994) The numerals on top of the diagram indicate the conventional chordate paralogy group of *Hox* genes (SCOTT, 1992).

Source MNHN, Paris

If the scenario I present here had only a portion of the value of AKAM's in this regard, I would be fully satisfied But my main purpose is to describe an example of what can be done in the field, rather than to present a completely realized scenario

THE BODY PLAN OF CRUSTACEANS AND HEXAPODS

The body plans of Crustacea and Hexapoda show a great parallelism Both include unsegmented parts at each end, the acron and telson, and three groups of segments, or *tagmata*, in between The first tagma is the *head* The cephalic parts of crustaceans and hexapods are very similar to each other

The second tagma of crustaceans is called the *pereion* It includes a number of segments, extremely variable according to the various sub-classes or orders. Each pereionic segment bears a pair of ventral appendages This is a strong rule only very few and derived crustacean species do not bear appendages on *all* pereionic segments The number of pereionic segments, although quite variable, is never three The second tagma of Hexapoda is the *thorax*, always composed of three segments Each hexapod thoracic segment bears a pair of legs, hence the name The presence of the three pairs of legs is again a strong rule, loss of legs always being a derived state On the other hand, the structure, function and even the mere presence of wings or wing-like appendages, are quite variable and distinctive traits among Hexapoda orders

The third tagma of Crustacea is called the *pleon* Again, it is composed of a highly variable number of segments, ranging from none at all in Cirripedia to more than twenty in some Notostraca (*Triops)* Depending on the crustacean order, or sometimes even genus, the pleon may or may not bear appendages The third tagma of Hexapoda is the *abdomen*, composed of a fixed number of 11 segments Only Collembola and some Diptera have a reduced number of abdominal segments At the adult stage, the last two abdominal segments may be reduced The hexapod abdomen does not bear locomotory limbs.

In Crustacea, the male genitalia are always located on the segment that marks the border between the pereion and the pleon (the last pereionic segment, sometimes called the genital segment) The female genital aperture may be located in the same position, or more anteriorly, depending on the Order In Hexapoda the genitalia are always located in a posterior position in males probably primarily on the 9th or 10th abdominal segment, in females probably primarily behind the 7th sternum

Despite the differences just described, the gross partitioning of the segmentation pattern of both Crustacea and Hexapoda into three parts leads to the proposal that the three tagmata of these two classes are homologous structures, following the corresponding order along the anterior to posterior axis The homology between crustacean and hexapod heads leaves little doubt, keeping in mind that the exact number of head segments is still controversial, and that there are some characteristic differences between the cephalic appendages of Crustacea and Hexapoda The homology between the second and third tagmata is less obvious but up to now has been generally agreed upon, and it is common in most classical zoology textbooks to find the term "thorax" used as a synonym for the crustacean pereion, and "abdomen" for the pleon

PHYLOGENETIC RELATIONSHIPS

The monophyly of the Hexapoda is rarely contested Among Hexapoda the relationships of the various orders are in general agreed upon It is also the case for the basal groups, the

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relations of which are becoming better clarified thanks to recent advances in cladistic analyses (KRISTENSEN, 1991, 1995, MINET & BOURGOIN, 1986)

The situation is just the reverse for Crustacea some sub-classes are clearly monophyletic, such as the Malacostraca But the relationships between groups are still very uncertain Thus, it is difficult to decide which of the crustacean sub-classes or super-orders represents the sister group of Malacostraca These problems are probably related to the diversity of body plans among Crustacea, and to the deep ancestry of the stem taxa of each lineage Recent molecular phylogenetic studies have not shed light on this question (ABELE *et al.*, 1992).

Another major problem concerns the relationship between Hexapoda and Crustacea Myriapoda, Hexapoda and Crustacea together belong to a "Mandibulata" clade, distinct from the other arthropod classes, namely the extinct Trilobita and the extant Chelicerata The Mandibulata are generally assumed to be monophyletic The most "classical" view is to consider the Myriapoda as the sister-group of Hexapoda They could be united into a clade called "Tracheata", on the basis of several similarities interpreted as synapomorphies, including the uniramous morphology of the limbs and the presence of tracheae, hence the name

However, recent molecular phylogenetic studies have led to the hypothesis that Hexapoda would be more closely related to Crustacea than to Myriapoda (BOORE *et al.*, 1995, FRIEDRICH & TAUTZ, 1995, TURBEVILLE *et al.*, 1991) This would lead to the rejection of the clade "Tracheata" comprising Hexapoda and Myriapoda Although still controversial, this phylogenetic hypothesis is supported by morphological arguments, mainly based on the striking similarity between the patterns of wiring of the nervous system between Crustacea and Hexapoda, which differs from that of Myriapoda (WHITINGTON *et al.*, 1991; OSORIO *et al.*, 1995, OSORIO & BACON, 1994). In addition, certain traits, previously taken as synapomorphies grouping Myriapoda with Hexapoda, such as the presence and morphology of the trachea, are now considered as convergent adaptations to terrestrial life It has even been proposed that hexapods might be evolved from a crustacean ancestor, thus making the class Crustacea paraphyletic (FRIEDRICH & TAUTZ, 1995, NIELSEN, 1995, p 162) (For a contradictory view on arthropod phylogeny, see FRYER, 1996)

COMPARATIVE DEVELOPMENTAL GENETICS

The body plan of metazoans is under the control of a particular set of genes, the homeotic or *Hox* genes, highly conserved in structure and function in triploblasts at least Each *Hox* gene is a "master gene" or "selector gene" which determines, in a specific domain along the anterior posterior axis of the body, a particular morphogenesis programme during development *Hox* genes were first discovered in a hexapod, the fruit fly and favourite genetic model *Drosophila melanogaster* They are now known in a variety of insects Recent studies on the branchiopod *Artemia* (AVEROF & AKAM, 1993) and on two cirripede species, (E MOUCHEL-VIELH & J S DEUTSCH, unpublished) show that the crustacean ancestor possessed a complement of the same eight typical *Hox* genes as found in *Drosophila* and other insects (Fig 1) In particular, Crustacea possess clear orthologues of both *Drosophila Ubx* and *abdA* genes This is not the case in the annelid *Holobdella*, where the *Hox* genes *Lox2* and *Lox4* are closer to each other than to either *Ubx* or *abdA*, indicating that the *Ubx/abdA* duplication postdated the divergence between Annelida and Arthropoda (WONG *et al.*, 1995) Unfortunately, data from the single chelicerate studied until now, *Limulus*, do not permit a determination of the precise relations of

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orthology with the other arthropod homeotic *Hox* genes, because of the short length of the fragments cloned and of the tetraploidy of the *Limulus* genome (CARTWRIGHT *et al.*, 1993) Hence, it is not yet possible to assess whether the *Ubx/abdA* duplication is shared by all members of the phylum Arthropoda or by Mandibulata only (Fig 1)

The variation in body plan between Crustacea and Hexapoda cannot be attributed to a simple change in the number and or structure of the *Hox* genes However, striking differences are observed between the pattern of expression of homologous *Hox* genes between Hexapoda and Crustacea Each *Hox* gene is expressed in a specific domain along the anterior-posterior axis of the animal during development. The specific domain of activity of each *Hox* gene actually results from this spatial specificity of expression and from the combinatorial interactions between the *Hox* genes' products In *Drosophila* the four more "posterior" *Hox* genes are *Antennapedia (Antp), Ultrabithorax (Ubx), abdominalA (abdA)* and *AbdominalB (AbdB)* The specific domain of *Antp* is the thorax, the *Ubx* domain comprises the posterior half of the second thoracic segment, the third thoracic and the anterior half of the first abdominal segment, the *abdA* domain expands from the second to the fourth abdominal segments, and *AbdB* reigns over the most terminal abdominal segments Besides details, this pattern of expression and activity is highly conserved in all Insects where it has been studied to date The same pattern of expression, including the same anterior limit of the domain in the thorax, has been found for the *Ubx* gene in such different Insects as the dipteran *Drosophila*, which bears a single pair of wings, the orthopteran *Schistocerca*, which bears two wing pairs (KELSH *et al.*, 1994) , and the zygentoman (= thysanuran *sensu stricto*) *Thermobia*, which bears no wing at all (CARROLL *et al.*, 1995) In contrast, in the crustacean branchiopod *Artemia* the pattern of expression of these *Hox* genes is quite different *Antp, Ubx* and *abdA* are all expressed in the pereion (the so-called "thorax") while *AbdB* expression is restricted to the genital segments (in *Artemia*, the genitalia are located in the last two pereionic segments) (Fig 2) Our preliminary results on Cirripedia, where the female genital aperture is located in the first pereionic segment, support the idea that *AbdB* specificity is genital rather than far-abdominal (E MOUCHEL-VIELH & J S DEUTSCH, unpublished)

From these results, AVEROF and AKAM proposed a reconsideration of the homology between the different tagmata of the two arthropod classes (AKAM, 1995, AVEROF & AKAM, 1995) In Crustacea, the segment bearing the male genitalia always marks the border between the pereion and the pleon, while the female aperture may or may not be located more anteriorly The crustacean genital segment (or segments) would be homologous to the genital segments of Hexapoda, which are always far-abdominal (see above) The crustacean pereion would not be homologous to the hexapod thorax, but rather to the whole (thorax + abdomen) (see Fig 2).

WHAT ABOUT LEGS ?

Hexapoda are distinct from Crustacea by their terrestrial life The terrestrial life of some crustacean species and the aquatic life of some hexapods are clearly secondary derived states Moreover, they differ by the number of pairs of ventral appendages All hexapods have three pairs of legs, one pair on each thoracic segment Crustaceans never have three pereionic segments Most have more than three, with the only exception of Ostracoda Hence, most Crustacea have more than three pairs of legs

Source MNHN, Paris

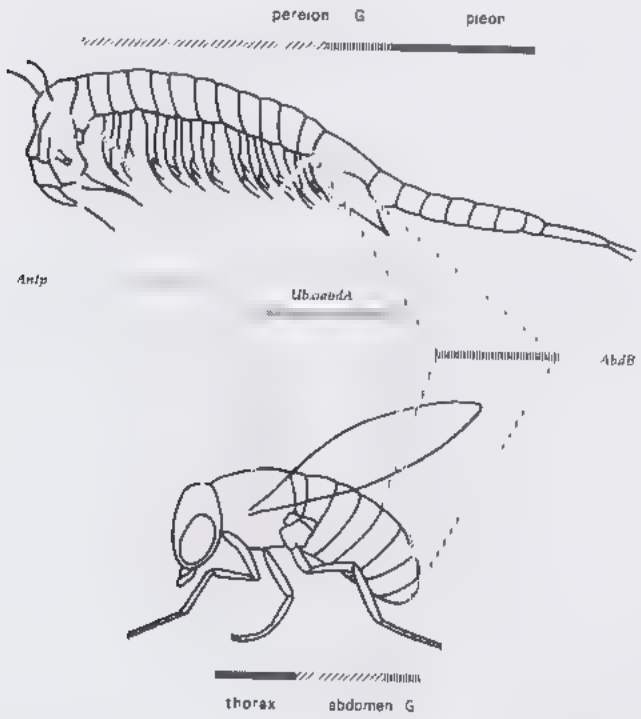

FIG 2 — The homology between the tagmata of Crustacea and Hexapoda according to AVEROF & AKAM (1995) The domains of expression of the *Hox* genes *Antp, Ubx, abdA* and *AbdB* are represented on a schematic drawing of *Artemia* (top) and *Drosophila* (bottom) For clarity sake, the *Hox* expression domains are represented on the body plan of an adult, although they have been observed at embryonic and or larval stages G genital segments the genital segments classically belong to the pereion in the Crustacea, and to the abdomen in the Insecta

It is logical to assume that the body plans of these two arthropod classes are related to their eco-ethological habits the crustacean ventral appendages are mainly used for swimming, while in a terrestrial environment it might be easier to use a small number of legs Other terrestrian mandibulates, such as Myriapoda and the terrestrian isopod crustaceans use quite different strategies for walking their pairs of legs are superabundant, but reduced in size, and homonomous Although probably primarily homomous, hexapod pairs of legs are not only reduced in number, they are also different from each other, in morphology, musculature and innervation This differentiation might well be under homeotic control Whether these two properties (number reduction and differentiation) are correlated is an open question Hence, this magic number of three thoracic segments and three pairs of legs in Hexapoda could be of adaptive value

Is it possible to reconcile the absence of legs on the hexapod abdominal segments with AVEROF and AKAM's hypothesis of homology between the hexapod abdomen and a part of the crustacean pereion?

In *Drosophila* the formation of thoracic legs and cephalic appendages requires the expression of the selector gene *Distalless (Dll)* In the thorax, the expression of *Dll* is repressed by the homeotic genes *Ubx* and *abdA* in the abdomen (VACHON *et al.*, 1992) Lack of expression

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of *Ubx*, as in the *bxd* mutant, yields to the formation of an additional pair of legs, a weird fly bearing four pairs of legs (BENDER *et al.*, 1983, and references therein)

The absence of abdominal legs in Hexapoda is not such a straight rule as it seems at the first glance Some insect nymphs and larvae possess abdominal appendages An example is given by the so-called "prolegs" of caterpillars In the butterfly *Precis coenia*, the formation of these prolegs has recently been studied (PANGANIBAN *et al.*, 1994) In the embryo, the *Hox* gene *Antp* is unexpectedly expressed in a few patches of abdominal cells, where *Ubx* and *abdA* are no longer expressed This unusual *Antp* expression is correlated with the expression of *Dll*, and the formation of the prolegs (WARREN *et al.*, 1994) Hence, the presence of abdominal legs in lepidopteran larvae is under the same genetic control that operates in the formation of thoracic legs In that case, the change results from a subtle and localized variation in the pattern of *Hox* expression

Another case of abdominal appendages in Hexapoda is the presence of abdominal styli in adults machilids (Archeognatha) and silver-fishes (Zygentoma = Thysanura *s. s*) It is generally admitted that machilid styli represent a primitive trait "The abdominal styli of machilids are generally regarded as remnants of telopodites" (BITSCH, 1994) Fossils support the homology of these appendages with true "legs" The abdominal styli of several extinct species are composed of several articles (plurisegmented), sometimes followed by a claw This morphology is quite similar to thoracic legs (for a review, see BITSCH, 1994)

The role of *Dll* has been recently examined in two crustaceans, the branchiopod *Artemia* and the malacostracan *Mysidopsis* *Dll* is expressed in every branch of the developing limb, whether it is uniramous or biramous The mode of expression of *Dll* and probably its function, as known in the uniramous insect limb, thus also applies to biramous limbs In contrast to insects, in the pereionic limbs of *Artemia*, *Dll* is expressed in the same cells in which the *Hox* genes *Ubx abdA* are simultaneously expressed (PANGANIBAN *et al.*, 1995) The anterior limit of expression of *Ubx abdA* coincides in *Artemia* with the transition from maxillae to pereiopods, while its posterior limit in *Mysidopsis* corresponds to the transition from presence to absence of pleopods In addition, the anterior limit of its expression varies in different malacostracan species studied with regard to the number of pereiopods transformed into maxillipedes (PATEL, 1995) Three conclusions can be derived from these comparative developmental genetics analyses i) *Dll* plays the same role in the developing limb in Crustacea as in Insecta, and probably in other Arthropoda (PANGANIBAN *et al.*, 1995), ii) the *Hox* genes play a role in Crustacea as well as in Insecta in directing the morphological diversity of limbs, and iii) contrary to Insecta, *Dll* is not repressed by *Ubx abdA* in Crustacea, and probably not regulated by any *Hox* gene

EVOLUTIONARY SCENARIO

Summarizing these data, the following evolutionary scenario can be drawn

i) The gross pattern of expression of *Hox* genes in *Artemia*, *i.e.* expression of *Antp*, *Ubx* and *abdA* in the pereion, *AbdB* in the genital segments, and no expression of *Hox* genes in the pleon, is primitive

ii) Several times, various genetic mutations have occurred during the evolution of crustaceans, affecting the regulation of the *Hox* genes These produced changes in the limits of the expression

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domains of *Hox* genes They account, at least in part, for the morphological diversity of extant and fossil Crustacea

iii) One of these changes happened to make a distinction between the first three pereionic and following segments, thus creating a "thorax" and an "abdomen" within the pregenital region In addition, at the same time or subsequently, subtle differentiation between the three thoracic segments was generated These changes were fixed during the change from aquatic to terrestrial life

iv) *Dll,* the master gene in the morphogenesis of appendages, acquired the cis-acting regulatory sequences that are a target for the products of the *Hox* genes *Dll* thus became integrated within the panel of genes regulated by the homeotics As a result, the abdominal legs were repressed This does not imply any important change of the *Hox* genes themselves or of their regulation, accounting for the striking stability of the body plan within Hexapoda (including fossils)

The above hypotheses constitute a scenario, meaning that the temporal succession of the proposed events in the order i) to iv) is part of the hypotheses

TESTS OF THE SCENARIO AND OPEN QUESTIONS

Obviously, the present scenario needs more data in order to be supported, from both phylogeny and molecular developmental genetics

The first major open phylogenetic question is the relationship between Crustacea and Hexapoda Morphological, developmental and molecular data have to be collected and considered in phylogenetic analyses in order to help resolve this important question

As stressed above, the relationships between the different sub-classes and orders of the Crustacea need to be clarified, by collecting more developmental genetic information on more species Although quite powerful, and more and more facilitated by the technological advances of molecular biology, molecular genetics is costly in time and money It is of major importance that the species used as models in comparative developmental genetics should be selected on the basis of their phylogenetic position This would not impair in any way the critical requirement of independence needed for phylogenetic tests of an evolutionary scenario (see GRANDCOLAS *et al* , this volume) Once obtained, the developmental genetic data could be drawn on an independently derived phylogenetic tree

More specifically, it is necessary to address the following questions

To what extent does the pattern of expression of *Hox* genes as found in *Artemia* apply to other Anostraca, to other Branchiopoda, to other members of "basal" groups of Crustacea ("basal" taken here in the sense of "a lineage emerging early in evolution from the main branch")?

To what extent is this pattern different from what is observed in Malacostraca? Since the published data are incomplete, the differences reported could result from a difference in the stage at which the larvae of the two crustaceans *Artemia* and *Mysidopsis* were observed, rather than to a specific difference

If the difference between *Artemia* and Malacostraca is confirmed, it would be of extreme importance to look for the expression of *Hox* genes in the pereion and in the pleon in Crustacea representative of other "basal" groups, in order to address the question whether this difference

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correlates to greater development of muscles and nervous system in the pleon of Malacostraca as compared to other Crustacea

What is the pattern of expression of the *Hox* genes, and in particular *abdA Ubx*, in members of more "basal" hexapod groups, such as Archeognatha and Collembola, rather than the representatives in which it has been observed, *i.e.* in Zygentoma and Pterygota In other words, is this pattern an apomorphy of the clade Insecta, or could it be a extended to the whole Hexapoda class?

What is the pattern of expression of *Dll* in the larval abdomen of machilids, and is it submitted to the same *Hox* control as found in other hexapods?

CONCLUSION

In the present work, I have proposed an evolutionary scenario on the origin of Hexapoda, and suggested some guidelines to test it

Some readers may think that this type of "gross" evolutionary question is not worth dealing with I have used this question as an example to illustrate the approach But the same approach could be applied to a variety of evolutionary problems at any level of taxonomy In the Hexapoda, evolutionary scenarios based on comparative developmental genetics can be drawn about such questions as the origin of the pterygote radiation (see CARROLL, 1995, CARROLL *et al.*, 1995) or the origin of the holometabolous radiation (see DEUTSCH, 1996) More specific problems can also be addressed The number and morphology of sex combs, located on the first leg of males, is a differential character in Drosophilid species between *montium* and *melanogaster* groups Not surprisingly, this character is genetically determined a "montium-like" mutant of *D. melanogaster* has been isolated (F DOCQUIER, P SANTAMARIA, and J S DEUTSCH , in prep) This example illustrates that developmental genetics might have something to tell even at the sub-genus level

Enhanced interaction between phylogenetic analysis and evolutionary developmental genetics will not only provide more data to improve the robustness and/or consistency of the proposed phylogenetic trees, (evolutionary patterns) but in addition may give an estimation of the validity of evolutionary scenarios (processes).

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Linking Phylogenetic Systematics to Evolutionary Biology: toward a Research Program in Biodiversity

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"Les recherches d'histoire naturelle, même celles qui ne semblent être que de pure et de vaine curiosité, peuvent avoir des utilités très réelles, qui suffiroient pour les justifier auprès de ceux mêmes qui voudroient qu'on ne cherchât que des choses utiles, si avant de les blâmer on avoit la patience d'attendre que le temps eut appris les usages qu'on en peut faire"

(DE REAUMUR, 1719)

As a matter of epilogue for a volume dealing with comparative studies, and although we hope that the reader is (now) convinced of the usefulness of phylogenetics, we would like to conclude in retrospection with the situation of systematics among life sciences

This situation may be explained in the frame of evolutionary biology, because systematics deals with the study of organisms with regard to their natural relationships which are evolutionary patterns Although self-evident, this matter of fact is often neglected This oversight as well as some current received wisdoms concerning systematics must be exposed because they impede the realization of major syntheses and integrated studies in biological sciences

Life sciences seem now on turn to be revolutionized by their two extremities On one hand, biological processes are more and more thoroughly analyzed using molecular techniques and a single organism now includes virtually hundreds of wide research fields On the other hand, we better and better understand how to compare and infer relationships between organisms using

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modern phylogenetic methodology Advances in these two extremities are the most recent manifestation of a long-lasting epistemological dichotomy, general biology versus comparative biology, "Investigations can seemingly inquire about either the uniformity of life or the diversity of life, aspects that can be referred to as general biology and comparative biology, respectively" (NELSON & PLATNICK, 1981).

Advances in general biology seem quite gradual at least with the continuous addition of new technical means and conceptual tools, and the discovery of new biological structures, from the organism to the cell, to the chromosome and to the gene and biomolecule The increase of knowledge in this gradualistic perspective is indeed exponential

Advances in comparative biology have been more punctuated with the recent and definitely new phylogenetics elaborated according to HENNIG's (1950, 1966) methodological principles, following former conceptions dating back at least to eighteenth century These principles brought a total revolution in the definition of relationships, turning down both intuitive classifications and unproper similarity algorithms Organisms may now be compared with a clear reference to their phylogenetic relationships *i e* to their evolutionary history – and not only as different items vaguely placed in an intuitive or misleading classification.

The main challenge of the next decades will be to unify biology by the implementation of a research program aimed at connecting general and comparative biology (Fig 1) It would not be sound to only analyze and understand more and more processes in a few unrelated organisms (*e.g. Drosophila*, *Rattus*, *Homo*, *Arabidopsis*, etc), or to only produce tens of phylogenetic trees and classifications for little-known organisms (KELLOGG & SHAFFER, 1993) This challenge will probably suffer resistance from *a priori* anthropocentric and utilitarian-centered views which

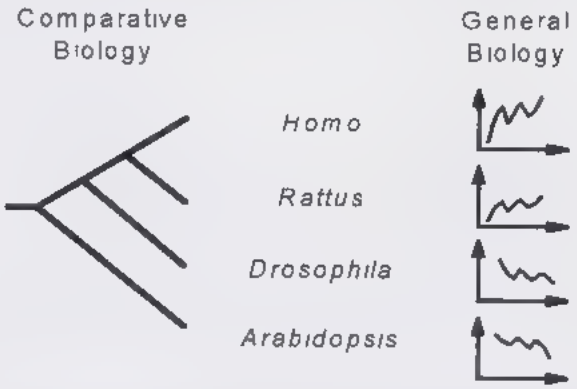

FIG 1 – Connecting general and comparative biology in a same research program will make necessary to take into account that few model organisms are studied by general biology and that they should be combined in the frame of phylogenetic relationships to be fully interpretable in the light of comparative biology and evolution

have always been very common in science and have tried to constrain its development in a way detrimental to *both* its usefulness and the increase in knowledge (*e g* DE REAUMUR, 1719)

The famous aphorism "nothing makes sense in biology, except in the light of Evolution" (DOBZHANSKY, 1973) is highly significant in this respect We may paraphrase nothing makes sense in biology, except in the light of a phylogenetic system which permit to consider all

Source MNHN, Paris

processes in a complete historical perspective This is true indeed for all studies in biology, even if some researchers often do not fully realize that their conclusions totally rely on the concept of phylogenetic relationships for example, many hypotheses of evolutionary convergence are commonly accepted for two structures, two states or two processes without recognizing that these hypotheses can *only* be substantiated by a genuine phylogenetic reference – we mean they cannot be said substantiated by an old and intuitive classificatory scheme

STUDYING EVOLUTION IN POPULATIONS OR EVOLUTION IN CLADES?

From the beginning, Evolution was perceived as a theory which needed to be substantiated not only according to the observation of patterns but also according to assumptions about processes at work (PERRIER, 1886) The promoters of the idea of evolution imagined processes such as spontaneous generation and inheritance of acquired characters (LAMARCK, 1809) or, later, descent with modification and natural selection (DARWIN, 1859) to explain the origin of hierarchical, inclusive and ordered patterns they observed

The rise of genetics and ecology at the beginning of our century confirmed the existence of descent with modification and promoted natural selection as a major process in evolution Evolutionary biology became a science of process and interest for tokogenesis replaced interest for phylogenesis (TASSY, 1991) Evidence for this fate is found in the assertion of some evolutionists who have argued that the great epoch of systematics or phylogeny, in terms of discovery, is the past, dating back to the eighteenth century, when the binominal nomenclature

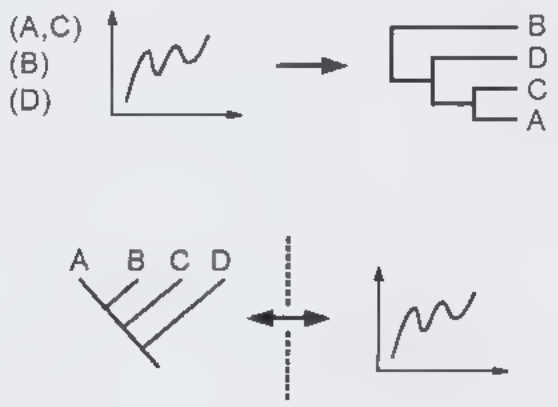

FIG 2 — The first scheme above summarizes the former procedure of explanation in evolutionary biology (in an hypothetical group [A, B, C, D]) processes (*e g* selective regime reaction norm, heritability) and traditional taxonomic levels were used to explain away patterns The second scheme beneath summarizes the procedure of phylogenetic tests of evolutionary scenarios both patterns in clades and processes in populations or organisms are actually studied, and the results are compared in a genuinely heuristic way

and then the idea of evolution appeared (MAYR, 1982) The concept of natural selection, now central to present evolutionary biology, has put the patterns in the shadow (ELDREDGE & CRACRAFT, 1980), although the main promoter of natural selection, Charles DARWIN himself, clearly acknowledged the important duality of pattern and process (DUPUIS, 1984, 1992)

Source MNHN, Paris

In this context, evolutionists focused on processes which were either extrapolated to explain patterns in *ad hoc* narrations as nicely emphasized by ELDREDGE & CRACRAFT (1980), or were combined with patterns in a same *synthetic* – we mean artificial and pleasant explanation of evolution (*e.g.* MAYR, 1963) (Fig 2, above) From this point of view, many evolutionary studies were carried out on organisms and populations since the beginning of the century These studies obviously focused on the processes existing within organisms and populations and extrapolated them back to the past macroevolutionary events BROOKS & MCLENNAN (1991) concluded from a fair retrospective that an eclipse of true historical studies occurred in ecology and ethology because of lack of phylogenetic and comparative studies Indeed, most historical studies were carried out by extrapolation and failed to analyze the past This assertion does not concern paleontological studies which deal *per se* with the past and suffer from another kind of extrapolation, the hypotheses of actualism (NEL, 1997 in this volume)

Extrapolation of our knowledge to past and unknown phenomena is certainly an heuristic means to propose provocative hypotheses but it is not a fair way to validate these hypotheses Extrapolation is in itself a model comprising not only factual knowledge but also many unwarranted hypotheses (mainly hypotheses by analogy from the present to the past) Thus, we need to study evolution both in clades and in populations Evolutionary biology must not be restricted to the study of only one of these fields, on pain of either ignoring the diversity of processes and their respective roles, or being unable to generalize the role of well-known processes to explain the diversity of life in itself

THE NATURE OF SYSTEMATICS AND SOME RECEIVED WISDOMS

Systematics is useful in evolutionary biology because it is central to studies of evolutionary patterns But today it carries certainly an odd image in life sciences After a great period of discovery and importance a hundred of years ago, it has been considered afterwards by many people as a marginal discipline in life sciences, a kind of narrative task whose goal is seen as to give unpronounceable latin names to as many species as possible, thus perceived by many researchers as "not modern science, not good science" In this perspective, the systematist would be only bound to identify specimens provided by other scientists who need names, and some have added nowadays to the basic requirement - latin names – , a classification or a phylogenetic tree

Let us abandon this incomplete image In systematics as in any other science, data must be collected first (field sampling of organisms), they must be described and analyzed (description of taxa and phylogenetic inference), and interpreted (evolutionary histories drawn from phylogenetic trees) It is this whole set of activities which can be used to answer scientific questions concerning evolution Systematics does not only provide people with names and classifications – which are obviously useful but also with a reference system in evolution (phylogenetic trees) The reference system itself is not only a system for storing and retrieving information concerning names, but also a very powerful means to draw evolutionary histories for traits of interest (ELDREDGE & CRACRAFT, 1980)

Systematics which was and which still is the science of evolutionary patterns has been revitalized these last decades by methodological advances (HENNIG, 1950, 1966) it can now generate refutable assumptions about evolution, which can be freed from evolutionary models derived from population studies Modern systematics is independent from these evolutionary models because it only uses the principle of descent with modification (the minimal concept of

Source MNHN, Paris

Evolution) to explain patterns, *i.e.* "aspects of the apparent orderliness of life" (ELDREDGE & CRACRAFT, 1980: 1). At the opposite, population or organismic studies search for processes, "mechanisms that generate these patterns" (ELDREDGE & CRACRAFT, 1980: 1).

This is the opportunity to reconcile both approaches in the study of evolution. Patterns and processes must be studied in their respective domains: clades and populations; and be compared afterwards to derive better-corroborated hypotheses about the past (Fig. 2).

In this perspective, several received wisdoms may be found in the literature that obscure the actual role of systematics in today life sciences. These received wisdoms must be denounced to fully allow integrated studies of evolution to develop, using systematics and population biology.

Is there still something to search for at the taxonomic-organismic level?

Many speculations have been produced during these last years about the number of extant species. Whatever may be the number of unknown taxa, it is obvious that many remain to be found. A better question should be answered: do unknown taxa represent uninformative replica of already known taxa or original and "useful" new taxa?

Most research scientists know that it is impossible to simply answer such a question "yes" or "no", because research is not a straightforward activity and wonderful results are often provided which differ from original conceptions or research fields.

A valuable answer to this question may be twofold. First, the frame of knowledge is not uniform: it is necessary to understand that past studies of biodiversity have not dealt randomly with faunas and floras for two or three centuries: some places or organisms were sampled over and over, some others were ignored. Important gaps in knowledge remain and must now be filled up, if one wants to have a good estimate of the contents of biosphere. Many scientific disciplines rely on such accurate estimates of these contents. Second, a prospective attempt may be done, using retrospectives of recent periods: many problems of biology have been solved thanks to an increase of knowledge of faunas and floras, for example when one taxon recently discovered gives up "the solution". It can be a fungus responsible for a local resistance against a dangerous illness in trees. It can be an insect species corroborating a famous hypothesis concerning the evolutionary process of social behavior. For all these reasons, it is clearly necessary to search for new taxa in faunas and floras.

However, these taxa must be searched for through modern and integrated field and laboratory studies by experts in a scientific discipline and in a group of organisms (recording ethological, ecological, biochemical, etc. information), and not essentially searched for in blind mass-collecting as practiced during the last centuries or even still recently. It is only in this way that scientific research can take rapidly benefit from systematics, also because the information relevant to the question the investigator wants to answer is sampled together with the specimen (CODDINGTON *et al.*, 1991).

Morpho-anatomy versus molecules?

New research fields opened out during these last decades in relation to outstanding technical advances in molecular biology. Consequently, enthusiastic professions of faith appeared which predicted revolutions in the understanding of Evolution. Newly available tools appeared especially powerful to people who simply forget that there already exist tools positively useful to

Source: MNHN, Paris

reconstruct phylogenies and that already allow most available and robust phylogenetic hypotheses to exist (morphology, anatomy, histology, etc), as shown by the retrospective of SANDERSON *et al* (1993) For example, for the period 1989-1991, more than 40% of published phylogenies in high-standard journals were based on morphology

It is still very common to read such kind of unfounded assertions. "A second reason for the surge of interest in comparative studies is that phylogenetic relationships among extant organisms are being estimated with increasing precision, largely as a result of advances in molecular biology" (HARVEY *et al.*, 1996) or "During the last decade, [ ] Together with improved phylogenies based on, for example, molecular sequence data, DNA hybridization, and other molecular methods [ ], it will lead to a dramatic increase in the power of comparative studies for clarifying selection and evolution " (ANDERSSON, 1994) This plea is however true for the organisms which are by themselves structurally closer to molecules than to multicellular organisms (viruses,.)

As emphasized by WAGELE & WETZEL (1994) or PHILIPPE (1997), molecular data cannot be the "only" or the "best" data which can be used to infer phylogenies, *because these qualifiers do not make sense* a priori *in science* (NELSON, 1994) Assertions of *a priori* explanatory contribution of any data set are misleading For example, STEARNS (1992) asserted "Two key advances were HENNIG's (1950) introduction of phylogenetic systematics (also called cladistics) and molecular systematics, where the non-transcribed part of the genome provides data independent of the confusing changes that evolution can produce in phenotypes" The dichotomy genotype/phenotype has nothing to do with phylogenetic methodology For inferring phylogenies, *heritable* characters must be used whatever their very nature How could we know *a priori* that evolution has produced more confusing changes in phenotypes than in genotypes in our case study? The value *a priori* of these characters is by definition unknown each time and cannot be extrapolated from one case study to another one

In this perspective, molecular data are obviously very welcome to join the pool of other data – which needs to be as large and relevant as possible and can help us in a number of cases to propose corroborated phylogenetic hypotheses (WENZEL, 1997 in this volume)

However, it is especially important not to throw out the baby with the bath water, and not to abandon morpho-anatomical studies and to shift completely toward molecular studies *Both* studies are obviously necessary and complementary because we need to understand how both morpho-anatomy and genome evolved, because we need to combine reasonably independent sets of data and because we need the largest sample of data possible

Are comparative studies too much speculative?

Many evolutionary biologists describe only the processes presently at work in populations This may lead one to believe that only direct or experimental observations can be used to understand how evolution occurred because other observations such as comparative hypotheses the phylogenetic patterns may seem *a contrario* too circumstantial In this context, *extrapolations* to the past evolution seem often correctly substantiated to many people, if they are based on such present observations

The key-word in this point of view is "extrapolation" The most careful studies of present-day processes must extrapolate to the past to generalize their results we are unaware of one study of populational or organismic evolutionary biology which does not make assumptions to

Source MNHN, Paris

extrapolate to past evolutionary events This is indeed a most interesting and significant part of these studies, but the least substantiated

As emphasized by one of us in the preface of this volume, these extrapolations are conjectural and may become either sound hypotheses or gratuitous speculation, depending on their refutability Extrapolations can be refuted only if some independent patterns exist which allow to test them This test is feasible using phylogenetic patterns, which do not depend on intentional assumptions of evolutionary processes Phylogenetic patterns are the less gratuitous and the more refutable reconstructions of the past, because we only need for inferring them the basic hypothesis of descent with modification Interaction between studies of present-day processes and studies of past patterns can provide us with well-corroborated results

In conclusion, comparative hypotheses are not specifically speculative Indeed, they prevent evolutionary hypotheses from being too much speculative

Too many model organisms in too many studies?

To the eyes of practicioners of general biology, the plethora of taxonomic groups used by comparative biologists may be taken as an impossible scientific challenge and an unreasonable scattering of model organisms This point of view is obviously related to an idiosyncratic perception of science by people who need to develop scientific studies in one model because of substantial technical feasibility constraints (KELLOGG & SHAFFER, 1993) For example, developmental genetics deals with very few organisms (DEUTSCH, 1997 in this volume) Comparative biology has not to cope with these limitations On the contrary, comparisons by *homology* and by *analogy* are the very nature of comparative biology (NELSON, 1970, LORENZ, 1974) It is thus essential to increase the number of sampled clades used in comparative biology to test the generality of evolutionary patterns as far as possible But these models must be carefully chosen to answer the questions of interest It is not the number but the *a priori* quality of these models which has to be controlled (GRANDCOLAS *et al.*, 1997 in this volume)

Is the time scale of evolution compatible with phylogenetics and the sampling of biodiversity?

Famous studies of processes in populations (*e g.* DOBZHANSKY, 1970, WHITE, 1954) have tied down the idea that evolution is generally rapid, except for a few relict species Thus, sampling the biodiversity may be seen as an impossible challenge also for this reason what is the need for describing species less rapidly than they appear and disappear?

First, some biologists studying processes have themselves considered that species may be relatively long-lived, for example, DOBZHANSKY *et al.* (1977) cited estimates ranging between 50,000 years and 1,000,000 years Actually, many paleontological works have shown that present-day taxa may be very ancient or that, more generally, species may be relatively long-lived (for example, in insects, BRUNDIN, 1988, MATILE, 1990, 1997 in this volume, or in mammals, JAEGER & HARTENBERGER, 1989) Specifically, paleontology and comparative biology can show that the preconceived idea of necessarily rapid evolution is not generally true biogeographers and paleontologists have documented that the present faunas include both recent and ancient stocks, the most ancient dating back to the Cenozoic or even to the Mesozoic This is another example of misconceptions related to the only care of studies in general biology

Generalizations may thus be false either because they are based on too few independent case studies or because they are based on *a priori* and totally speculative ideas For example,

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particular models of evolution have been hypothesized under which some kinds of phylogenetic reconstructions are flawed (*e.g.* FELSENSTEIN, 1978, FRUMHOFF & REEVE, 1988), but these models of evolution are impossible to assess *a priori* right or wrong in a particular case, *i.e.* prior to any phylogenetic reconstruction (see for example CARPENTER, 1997, and GRANDCOLAS *et al.*, 1997, both in this volume). So their usefulness is nil

Biodiversity: what's in a word?

The concept of Biodiversity has nowadays a central position among scientific and political debates and policies There are many different claims for its restriction to an organismic versus a genetic or a molecular significance, or to process versus pattern viewpoint In our mind, the origin, maintenance and loss of biodiversity will be respectively better-known or prevented thanks to a high "biodiversity" of research scientists and scientific approaches

The "biodiversity" of approaches should respect the natural hierarchy of research fields What could be the meaning of genetic or molecular approaches without any population or organismic approaches (BARBAULT, 1988) and what could be the meaning of all these approaches without any phylogenetic framework? We could add, without any appropriate and recently corroborated phylogenetic framework

As argued by DUPUIS (1992), this framework is not only a database system in which taxa and processes are stored and may be retrieved This last view has been popularized because it emphasizes the most immediate and technological utility of systematics' identification and classification No more systematists, no more identifications or classifications! This could be the immediate prejudicial effect that disappearance of systematics could have (*e.g.* WILSON, 1971; S A. 2000, 1994)

But the phylogenetic framework is also an explanatory system which permits to test the macroevolutionary extrapolation of present-day processes and an heuristic system which permits to propose new research directions (JANVIER, 1984, PACKER, 1997 in this volume) In this way, phylogenetic systematics is a federating discipline and framework which connects all parts of life sciences and generates a research program in biodiversity.

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